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ダイズシストセンチュウ (*Heterodera glycines*) に関する研究

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Studies on the Soybean Cyst Nematode *Heterodera glycines*

By

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(JAF)

本報告には元当場勤務(現農林省農業技術研究所勤務)農林技官一戸稔の提出に係るダイズシストセンチュウ (*Heterodera glycines*) に関する研究成績を登載する。

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I. Introduction

It has only been within the last two or three years that the economic importance of plant parasitic nematodes has aroused the interest of most agronomists and farmers in this country, in spite of the fact that nematodes have been damaging plants to a considerable extent. As is well known, it is one of the major projects of the Ministry of Agriculture and Forestry to improve the productivity of the so-called "field crops" such as vegetables, potatoes, sugar-beet, beans and so forth. Since soil nematodes constitute an important part of the soil-borne pests of those crops, the 5-year programme has been developed by the Ministry and designed to teach nematode bionomics, emphasizing the necessity of controlling nematodes in order to increase the soil productivity. In accordance with this programme, the "soil inspectors" who are now stationed in each prefecture of this country have been investigating soil nematodes on a nation-wide scale since 1959.

The nematodes which have been found so far in Japan include the root-knot nematodes (*Meloidogyne* spp.), the cyst nematodes (*Heterodera* spp.), and the root lesion nematodes (*Pratylenchus* spp.), all of which could be of major economic importance. These three genera of nematodes were recorded in Japan for the first time in 1895, 1915, and 1932 respectively.

The soybean cyst nematode, *Heterodera glycines*, was the only species of *Heterodera* found in Japan up to 1955, following which the oat cyst nematode, *H. major* was recorded by the author as the second species of *Heterodera*. The extensive soil surveys being made by nematologists, as noted above, have only just begun, yet it has become evident that there must be several additional species of *Heterodera* in Japan which appear to attack the roots of plants such as rice, clover, sugar-beet and peanuts etc.

The soybean cyst nematode has been known to be one of the most destructive pests of soybean, the plant being one of the most important and extensively cultivated leguminous crops in this country. Here, the damage to soybean plants by this nematode is briefly discussed. During the summer of 1952, the author participated in a cooperative survey

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on the nematode injury in the soybean growing area of Memuro, Hokkaido. Tokachi Province, in which Memuro is located, is supposed to be the present biggest soybean producing area in Japan, and Memuro has the largest soybean fields in Tokachi Province. In this survey every field where soybean grew was examined as to the degree of nematode infestation from the symptomatic conditions of plants. In addition to this, a similar survey was made covering the whole of Tokachi Province. The results of this survey showed that the average yield reduction of soybeans due to the nematode was found to be 41.3 per cent of that of non-affected plants. The total cropped area of land was 41,639 acres, of which 17,336 acres (41.6 per cent) were cropped with beans. Of all bean-cropped land, 8,707 acres (50.2 per cent) were found to be infested with the soybean cyst nematode. The estimated amount of damage to the soybeans caused by the nematode was 115,000,000 yen at the 1952 market price. The corresponding figures for the entire Tokachi Province were recorded as follows; 326,623 acres of cropped land, 134,102 acres (41.1 per cent) of which were planted or alloted for soybean culture and 37,611 acres (28.0 per cent) of which was found infested, causing an estimated loss of crop equivalent to 497,000,000 yen.

It has been frequently shown that affected soybean plants decrease in height and yield and heavily affected plants may grow to only one-third the height of normal plants. It is not uncommon that the yield at harvest is smaller than the quantity of seed sown. According to the agricultural statistics of 1957, Hokkaido produced 84,710 tons of soybean which corresponded to almost 20 per cent of the total produced in Japan. It can be said, therefore, that the most destructive nematode of all in Hokkaido is the soybean cyst nematode.

The soybean cyst nematode seems to be distributed only in the Asian countries until for the first time outside of Asia it was found in North Carolina, U. S. A. in 1954. Since then the distribution of this pest in the United States has been extending year after year, and it seems to pose a serious threat to the nation's soybean industry.

The author has been studying chiefly the morphology, bionomics and control of the soybean cyst nematode since 1949, most of which was done during the author's previous employment in the Hokkaido National Agricultural Experiment Station, Kotoni, Sapporo, Japan. Results obtained through these studies and investigations are reported herein. A brief preliminary report has however already been presented (ICHINOHE, 1959).

II. Common Names of the Disease, Plant Symptoms, and the Geographical Distribution

Common names of the soybean disease and the causal nematode According to ISHIKAWA (1916), the soybean disease in Niigata Prefecture was called "Tsukiyo-byô" (literally translated into "moon-night disease") apparently after the characteristic appearance of the diseased part of the fields which was likened to the full moon. There were evidently several different names in the local dialects at that time.

KATSUFUJI (1919) named this disease "Iwô-byô" (or "yellow dwarf disease") after the

characteristic symptoms of yellowish discolouration of affected plants accompanied by retarded growth of shoots.

It was proposed in 1958 that as a common name of the causal nematode, ***Heterodera glycines***, "Daizu-shisuto-senchû" or "soybean cyst nematode" should be used.

Symptoms of soybean plant affected by the nematode The "yellow dwarf" disease caused by the soybean cyst nematode appears in fields toward the middle of July in Hokkaido, approximately two months after seeding.

The characteristic symptoms of the plant are severe retardation of growth, stunting, and yellowish appearance on the above-ground parts of the plant. The foliage of the affected plant turns yellow in colour and falls off early. The plant sometimes bears only a few flowers and a few seeds, which are small in size and inferior in quality compared to the normal seeds. The roots of the affected plants develop many more lateral rootlets and possess, in most cases, many fewer root nodules than those of healthy plants.

In the field the disease appears at first in certain limited areas, more or less circular or less frequently irregularly shaped patches of discolouration. These patches become more distinct within the subsequent few weeks and then spread as the season proceeds. In most cases it is only after the distinct patches have appeared that the farmers realize the presence of the causal nematode in the field. In case soybeans are successively planted on the same infested land, the patches expand rapidly and will completely cover large fields within three or four years. Since these yellowish and dwarfed plants in the field are conspicuous, the occurrence of the disease can be easily recognized without walking into the field, even from a distance. Generally speaking, symptoms are most noticeable on seedlings though they appear during any period of the plant growth.

Geographical distribution of the nematode The known distribution of this nematode in Japan is Hokkaido-, Honshû- (mainland), Shikoku-, and Kyûshû Islands. Few reports on the detailed distribution of this nematode have made in these parts except from Hokkaido, probably because of the minor importance of the soybean crop.

According to ITO (1921), the soybean cyst nematode was discovered in four localities in the southern part of Hokkaido in or around 1920. The survey made in 1932 to 1935 by the Hokkaido Agricultural Experiment Station revealed 49 localities infested with this nematode. By 1958, more than 64 localities, all restricted to the southern part of Hokkaido, were found by the author to be infested. Almost all of the infested localities have soil types such as sandy, less-organic, or volcanic ash soils; these soils are prevailing in the soybean crop areas in Hokkaido.

YOKOO (1936) mentioned that this nematode had been observed in Korea.

NAKATA and ASUYAMA (1938) reported that the soybean cyst nematode was found in Manchuria (northern part of Red China). According to their report, the nematode was found at Khu-lan (Pin-kiang hsiu), Tsi-tsi-har (Helung-kiang hsiu), Tao-nan, Khi-shu-lien (Kih-rin-hsiu), where the damages to certain varieties of soybean were so serious that plants failed to grow at all and died early. They also stated that this disease did not seem to

be spreading very widely, as of 1936, as it was restricted to the districts mentioned above.

No reports on the occurrence of this nematode have been made in any other part of Asia up until 1955.

The first report of the occurrence of the soybean cyst nematode outside of the Orient was made by WINSTEAD, SKOTLAND and SASSER (1955) who discovered its presence in the southeast of North Carolina, U. S. A. in the summer of 1954. Since then, according to the survey conducted by Plant Pest Control Division, U. S. Department of Agriculture, the soybean cyst nematode, as of November 30th, 1958, has been found in seven States of east of North Carolina and the Mississippi Valley, viz., Arkansas, Kentucky, Mississippi, Missouri, North Carolina, Tennessee, Virginia. In these States 549 affected properties, totalling 22,714 acres of infested land, were discovered.

III. Studies on the Morphology and the Systematics of the Soybean Cyst Nematode

1. HISTORICAL REVIEW

The soybean cyst nematode was first recorded by HORI (1915). According to the report, HORI discovered the nematode on the roots of soybean plants sent from Shirakawa, Fukushima Prefecture, in the north-eastern part of the mainland, where the same disease had been observed for many years. He stated that this nematode is different from the root-knot nematode previously known in Japan at that time and asked S. UCHIDA to identify it. In accordance with S. UCHIDA's identification, HORI referred to the nematode as a species closely related to *Heterodera schachtii* which had been well known in Europe as the causal nematode of "sugar-beet sickness". HORI adds in his paper that it is necessary to compare this nematode with *Heterodera göttingiana* known in Germany, as this latter species selects the pea as host plant.

In the next year, ISHIKAWA (1916) reported that in Niigata Prefecture, in the north-central part of the mainland, the soybean disease had occurred for many years and it was thought to be caused by the same organism as the one HORI (1915) reported.

The soybean cyst nematode was reported from Hokkaido by KATSUFUJI (1919) and then by ITO (1921). According to ITO (1921), this disease had been observed on soybean for more than 10 years, and the nematode was thought to be the same species as the sugar-beet nematode, *Heterodera schachtii*, judging from the morphology of females and larvae.

TANAKA (1921) reported that the causal nematode of the "moon-night" disease of soybeans found in Ibaragi Prefecture seemed different from the sugar-beet nematode, *Heterodera schachtii*.

FUJITA and MIURA (1934) reported the parasitism and the systematics of this nematode. They stated that from the results of a series of experiments on the range of host plants, the soybean cyst nematode may be a biological race of *H. schachtii* occurring in Japan, being similar to those which had been called the pea-, oat-, and potato-race of *Heterodera*

schachtii in Europe.

FRANKLIN (1940) raised some so-called biological strains (or races) of *H. schachtii* to the rank of a species after morphological studies on those nematodes. Since then, the specific status of the soybean cyst nematode has been so indistinct that most of the workers have referred to it as *H. schachtii*, while some workers viz., FILIPJEV and SCHUURMANS STEKHOVEN (1941), GOFFART (1951), YOKOO (1951), have considered it to be *H. göttingiana*.

In 1952 the author compared the soybean cyst nematode with specimens of the other species of *Heterodera*, and described it as a new species under the name *Heterodera glycines* (ICHINOHE, 1952). There is no doubt that the nematode found on soybeans in Honshu (mainland) and Hokkaido is identical.

Since the soybean cyst nematode was found in the U. S. A. in 1954 for the first time outside of the Orient, H. HIRSCHMANN (1956) made a detailed comparison of this nematode with the clover cyst nematode, *H. trifolii*. She made clear the distinct morphological differences between the two species in the second-stage larvae.

2. MATERIALS AND METHODS

Studies on the morphology of the soybean cyst nematode described herein were made mostly during the period from 1950 to 1952 and part of these studies were previously reported by the author (1952, 1955).

The nematode materials used throughout the investigations were obtained from cultures of soybean plants grown in nematode-infested frames at the Hokkaido National Agricultural Experiment Station (Fig. 1). These frames were filled with the soil originally brought from the nematode infested area in Tokachi Province, Hokkaido. The soybean variety "Ishikari-shiro No. 1" and other varieties were seeded in these frames.

Various life stages of the nematode inside the root tissue were obtained by means of the procedure recommended by GOODEY (1937). For this purpose the roots of soybean

Fig. 1. The nematode-infested concrete frames used for the experiments at the Hokkaido National Agricultural Experiment Station.

seedlings were washed free of soil, stained by dipping them in hot lactophenol with acid fuchsin for one minute, dehydrated with ethylene alcohol and then cleared in clove oil. In some cases, instead of the dehydration process, the roots were transfered from hot acid fuchsin-lactophenol to plain lactophenol in order to clear them without dehydration. The roots of soybeans which were fixed in 5 per cent formalin, were also utilized for the collection of the nematode materials. Adult males were obtained in abundance by keeping the roots, infested by adult females, in a moistened glass chamber for two or three days at 25°C, and then rinsing and sieving.

3. MORPHOLOGICAL DESCRIPTION OF LIFE STAGES OF THE SOYBEAN CYST NEMATODE

Egg The egg is originally somewhat barrel-shaped, but as the embryo increases in length it becomes more slender and oblong with round ends and slightly concave sides. The egg is 107 micron in length and 43 micron in width. The egg measurements are given in Table 1.

Table 1. Measurements of the eggs of *Heterodera glycines* (in micron).[1]

Length			Width		
Maximum	Minimum	Mean	Maximum	Minimum	Mean
118	95	107	47	39	43

1) Based upon 100 eggs (10 eggs from each of 10 cysts).

First-stage larva When the embryo fully elongates and reflects inside the egg-shell, it develops into the first-stage larva. The first-stage larva soon molts to the second-stage larva still inside the egg-shell. Therefore, it is the second-stage larva that hatches out of the egg and this is similar to what RASKI (1950) described and illustrated with the eggs of the sugar-beet nematode, ***Heterodera schachtii.***

The first-stage larva has internally a very poorly developed alimentary canal, and in the head region a slightly differentiated prorhabdion is visible. The first-stage larva moults inside the egg-shell and it is usually very difficult to recognize, but the appropriate stage of this larva often clearly shows the molted cuticle in its head region. If the larva is forced to come out of the egg-shell by placing eggs on a glass slide and gently pushing the cover glass, the molted cuticle of the first-stage larva is left inside the egg-shell as the second-stage larva emerges.

Second-stage larva The second-stage larva is worm-like in shape, free-living, and infectious. The larva penetrates the root tissue, molts inside the tissue, and finally becomes the third-stage larva. The second-stage larva having survived in soil or water for several weeks demonstrates a sign of starvation by evacuating the intestine; such larva is easily distinguished from newly hatched larva.

The second-stage larva varies in length from 437 to 504 micron with an average of

471 micron, and 18.0 to 18.5 micron in width with an average of 18.3 micron. The length of the second-stage larva of the soybean cyst nematode falls into the "medium" group according to FENWICK and FRANKLIN (1951) measured by the standard technique as is shown in Table 2.

Table 2. Length of the second-stage larvae of *Heterodera glycines* measured by the technique of FENWICK and FRANKLIN (in micron).[1]

Cyst No.	Maximum	Minimum	Mean
1	486	437	460
2	484	447	465
3	504	452	489
4	499	452	473
5	494	447	469
6	471	437	456
7	491	463	476
8	499	465	486
9	504	447	471
10	494	437	460

1) Based upon 10 larvae from each of 10 cysts.

The annulations of the cuticle are moderately coarse. The lateral field is marked by four longitudinal lines which start at a position near the stylet-knobs and extend to about the middle of the tail. The lip region, definitely set off by constriction, is almost hemispherical, being 4.5×8.9 micron in size, and bearing three striations. As seen *en face*, the lip region consists of six radially arranged lips, of which the lateral ones have slit-like amphidial openings and they are a little smaller than the others. The cheilorhabdion is heavily sclerotized. The stylet is about 23 micron in length (M=23.1 micron), with anterior protruding basal knobs. The median bulb of the oesophagus is 16.5×10 micron in size; the distance from the anterior end of the body to the posterior end of the median bulb is about 69 micron. The orifice of the dorsal oesophageal gland is located about 4 micron in a posterior position to the basal knobs. The glandular terminal bulb of the oesophagus overlaps the intestine. The excretory pore is situated ventrally 92 micron from the anterior end. The anus is located from 42 to 47 micron (M=45.0 micron) from the tail tip. The genital primordium is ovoid in shape, located ventrally and slightly posterior to the middle of the body, being 15×8.5 micron in size, and composed of two protoplasmic masses, each of which has a nucleus. The body cavity extends up to 20 micron in length posterior to the anus. The phasmid is visible as a minute glandular structure opening at the middle of the lateral field and located 10 micron, or five to six annules, posterior to the anus.

Third-stage larva The third-stage larva is always found inside the root tissue. It

is not worm-like but sausage-shaped. The sexes can be differentiated as the genital primordium of this stage of larva develops.

The third-stage larva varies from 0.32 to 0.39 mm in length and 0.048 to 0.084 mm in width. No differences between sexes in the size of the larvae are found. The cuticle has no distinct marking throughout the body except for about ten narrow annules on the anterior end. The head region is not so complicated as that of the second-stage larva, but simply has narrow cross annulations. The stylet is much less robust, being from about 10 to 15 micron in length, with small basal knobs. The median oesophageal bulb is well-developed, being much larger than that of the second-stage larva, and consistently larger in females than in males. This is also the same in the subsequent larval stage. The dorsal gland is well-developed in both sexes; the female larva generally has one a little larger than that of the male. The rectum is distinct.

The development of the genital primordium is most conspicuous in the third-stage larva showing a great deal of variations in shape and length. The genital primordium is at first oblong, being 30×20 micron in size, and then it becomes elongate.

In male larvae, the upward elongation of the primordium becomes reflexed and the downward elongation reaches a point slightly anterior to the rectum. In female larvae, both ends of the genital primordium elongate upward, forming eventually two ovaries in the subsequent life stage.

Out of many individuals of the third-stage male larvae examined, the author encountered a single specimen which has internally a cellular lateral string beneath the cuticle. These string cells seem to be the same as the one RASKI (1950) described in *H. schachtii.* He states that "the nature of these cells is not known but it is possible that they are in some way related to the lateral chords in the developing male".

Table 3. Measurements of the third-stage larvae of *Heterodera glycines* (in micron).

Individual No. and sex	Body		Distance from the anterior to excretory pore	Median oesophageal bulb		Length of tail
	Length	Width		Length	Width	
1 (M)	390	48	87	19.5	17.5	17.5
2 (M)	329	65	78	21.5	17.5	11.0
3 (M)	325	71	80	19.5	17.5	13.0
4 (M)	351	65	74	21.5	17.5	11.0
5 (M)	364	74	91	21.5	17.5	11.0
6 (F)	355	52	80	24.0	17.5	13.0
7 (F)	329	61	78	26.0	19.5	15.0
8 (F)	360	59	82	24.0	21.5	9.5
9 (F)	386	71	87	26.0	24.0	13.0
10 (F)	351	84	82	26.0	26.0	13.0

The measurements of third-stage larvae are given in Table 3. The third-stage larva molts and develops into the fourth-stage larva inside the root tissue.

Fourth-stage male larva The fourth-stage male larva appears to cease feeding. The process of molting by the third-stage male larva is quite different from that of any other stage of larvae. The larva begins its molt by shrinking the body inside the third-stage larval cuticule; this is followed by an elongation of the body, becoming coiled inside the cast skin. In many cases, the first reflex of the larva occurs at the posterior part of the body. The fourth-stage male larva continues to elongate and finally forms three coils becoming typically worm-like and similar to the adult male in shape.

The cuticle has no distinct markings. The lip region, stylet, and oesophagus begin to develop into those of the adult male. The anus is indistinct. The developing testis reach the rectal region within this stage appearing continuous with it. In the posterior region differentiation of the spicules occurs. The measurements of the fourth-stage male larvae are given in Table 4.

Table 4. Measurements of the fourth-stage male larvae of *Heterodera glycines* (in micron).

Individual No.	Body width	Cast skin (Third-stage larval skin)		Median oesophageal bulb	
		Length	Width	Length	Width
1	34.5	355	87	19.5	17.5
2	30.5	377	56	19.5	17.5
3	26.0	360	72	19.5	15.0
4	26.0	334	72	19.5	15.0
5	24.0	373	78	19.5	15.0

Fourth-stage female larva The fourth-stage female larva is typically flask-shaped, being very similar to the form of the adult female except that the former does not have

Table 5. Measurements of the fourth-stage female larvae of *Heterodera glycines* (in micron).

Individual No.	Body		Distance from the anterior to excretory pore	Median oesophageal bulb	
	Length	Width		Length	Width
1	360	152	61	29.5	26.0
2	347	165	78	34.5	24.0
3	355	178	108	32.5	27.0
4	373	186	100	34.5	28.0
5	399	182	82	30.5	30.5
6	355	191	82	35.0	30.5

the protruding posterior end. The cuticle thickens throughout the body particularly at the posterior region. The stylet is not robust. The median oesophageal bulb is well-developed. The elongation of the genital primordium which started in the third-stage larva, is still observed in this stage developing into the structure which corresponds to the coiled ovaries in the adult female. The measurements of the fourth-stage female larvae are given in Table 5.

Adult male The adult male emerges from the third-stage larval cuticle, casting off at the same time the fourth-stage larval skin. The adult male is typically worm-like in shape, becoming the free-living form in the soil, and demonstrates very little morphological specialization to the parasitic form. It is not unusual to find a living male inside the gelatinous egg-sac attached to the female, but little is known as to whether the males have a role in propagation of this species.

The adult male varies from 1.2 to 1.4 mm (M=1.33 mm) in length and 27 to 31 micron (M=28.6 micron) in width. The tail length varies from 1.7 to 5.5 micron (M=3.5 micron). The cuticle is 3.0 to 3.5 micron in thickness being composed of three layers, and the transverse striations on the cuticle are from 1.5 to 2.4 micron apart. The lateral field is marked with four longitudinal lines extending the length of the body and beginning as three lines anteriorly but soon increasing to four at the position posterior to the base of stylet. Posteriorly the four lines separate slightly as they extend around the blunt tail, and the two lateral fields meet at a mid-line on the tail. The head region is hemispherical, set off by constriction, being 6.2×11.3 micron in size, and bearing four or five annules. In face view the head region has six radially arranged lips, of which the lateral lips have slit-like amphidial openings more or less smaller in size than the others. The

Table 6. Measurements of the adult males of *Heterodera glycines* (in micron).

Individual No.	Length	Width	Length of stylet	Length of oesophagus	Distance from the anterior to excretory pore	Length of testis
1	1358	28.2	26.7	165	147	733
2	1300	27.7	26.0	182	142	633
3	1242	27.3	26.7	173	157	725
4	1350	28.2	26.7	173	145	700
5	1392	29.5	25.0	169	150	742
6	1258	28.2	26.7	173	150	725
7	1367	29.0	26.5	178	148	742
8	1383	30.3	26.5	180	143	708
9	1300	27.7	26.2	160	142	750
10	1392	29.5	26.0	178	143	692

cephalic framework is heavily sclerotized. The stylet is 26.8 micron in mean length with lateral to anterior protruding knobs. The oesophagus, from 160 to 180 micron (M=173 micron) in length, is divided into three parts, viz., a muscular bundle posterior to the stylet, median bulb, and oesophageal glands. The median bulb is 19.4×12.8 micron in size. The dorsal oesophageal gland overlaps the intestine forming glandular lobe. The ducts of the oesophageal glands are rather conspicuous in many specimens. The orifice of the dorsal oesophageal gland is located about 4 micron posterior to the base of the stylet knobs, and two subventral oesophageal glands open into the lumen of the median bulb. The testis start at the middle of the body or slightly anterior. The spicules are slightly arcuate, being 34 micron long with bifid tips. A gubernaculum is from 11.5 to 12.0 micron (M=11.7 micron) in length. The excretory pore is funnel-shaped and located ventrally 144 ± 11.0 micron from the anterior. The phasmid is located from 2 to 8 micron (in most cases 4 to 6 micron) from the posterior. The measurements of the adult male are given in Table 6.

Adult female The adult female is not worm-like but swollen and lemon-shaped. The matured females appear outside of the root, and therefore they are visible to the naked eye. When a female dies the body wall hardens and becomes a sac for the eggs and referred to as a "cyst". In a single cyst there are several hundred eggs and each of the eggs contains a second-stage larva which is coiled inside the egg-shell.

The body is lemon-shaped with a short neck, from 0.07 to 0.10 mm long at the anterior end and with a prominent vulva to the posterior. The adult female varies from 0.47 to 0.79 mm in length and 0.21 to 0.58 mm in width. The female is white in colour at first and then turns yellow as the eggs develop. The newly developed female is coated with the often termed "subcrystalline layer" which persists on the brown cyst. The cuticle is thickened from 7 to 9 micron at the middle section of the body and from 9 to 11 micron at the neck and the posterior region. The cuticle is composed of three layers, the outer layer being marked by a rugose pattern except for several annulations on the anterior end. In face-view the head region does not bear six radial lips, but instead an hexagonal circumoral cuticular plate. The stylet is somewhat slender with posterior protruding knobs and 27.5 micron in mean length. The median oesophageal bulb is much larger than those of any other life stages, being 39.0 by 32.5 micron as the maximum size. The anus is located ventrally 75 to 90 micron from the middle of the vulva. The paired ovaries are coiled filling almost the entire body cavity, and open to the vulva at the posterior end. Various sclerotized structures surround the vulva. A gelatinous egg sac, roughly one-third of the body in size, is attached to the vulva and into which the embryonated eggs are deposited.

The number of eggs deposited in the egg sac varies from a few to about 200. This data is given in Table 7.

Table 7. Number of eggs produced by a single female of *Heterodera glycines* matured on the soybean plant.

Individual No.	Number of eggs in cyst (A)	Number of eggs in egg-sac (B)	No. of eggs produced by one female (A+B)	% ratio of no. of eggs in egg-sac $\left(\frac{B}{A+B}\right)$
1	476	12	488	2.5
2	184	44	228	19.3
3	304	119	423	28.1
4	256	131	387	33.9
5	346	218	564	38.7

Cyst The cyst is brown in colour and lemon-shaped. It is 699±60 micron in length, 490±54 micron in width, and 1.43 in ratio of length to width. The cyst wall is composed of two layers, of which the outer layer is marked by a rugose pattern and the inner layer by minute punctations which are usually arranged with a tendency to run in parallel rows, particularly on the part of the posterior region. The measurements of the cysts are given in Table 8.

Table 8. Measurements of cysts of *Heterodera glycines* (in micron).1)

Population No.	Length2)			Width			Ratio of length to width
	Maximum	Minimum	Mean	Maximum	Minimum	Mean	
1	808	640	726	589	438	523	1.39
2	850	614	698	547	387	475	1.47
3	791	614	695	564	370	494	1.41
4	800	623	705	555	429	503	1.40
5	757	606	687	572	387	473	1.45
6	774	589	683	547	404	460	1.48
7	808	572	709	547	370	478	1.48
8	808	555	711	581	354	513	1.39
9	741	600	675	572	421	482	1.40
10	808	564	708	581	404	492	1.44

1) Based upon 100 cysts (10 cysts from each of 10 populations).

2) Measurement excluding neck.

Counts were made to determine the number of eggs contained within a single cyst. From 95 to 478 eggs averaging 262 eggs per cyst, were counted from sampling 66 cysts in all which had matured on soybean plants. The data is given in Table 9.

Table 9. Number of eggs contained within a single cyst of *Heterodera glycines* matured on soybeans.

Number of eggs contained in a single cyst	Number of cysts which contain the number of eggs indicated in the left-hand column	
	Test "A"[1]	Test "B"[2]
less than 100	0	2
101～200	5	8
201～300	17	10
301～400	13	9
401～500	0	2
more than 501	0	0
Number of cysts examined	35	31

1) Test made in October 1949 with the cysts obtained at Obihiro, Hokkaido.
2) Test made in November 1953 with the cysts obtained at Shimamatsu, Hokkaido.

4. CONSIDERATIONS ON THE SYSTEMATICS OF THE SOYBEAN CYST NEMATODE

Systematic description of *Heterodera glycines*

Heterodera glycines ICHINOHE, 1952

ICHINOHE, M., Ôyô-Dôbutsugaku Zasshi, 17 (1/2), pp. 1～4, 1952.

Dimensions:

100 cysts Length 0.70 mm (s = ±0.060 mm)
Width 0.49 mm (s = ±0.054 mm)
Length/width 1.43 (range 1.20～1.61)
25 males Length 1.31 mm (s = ±0.098 mm)
a = 40, b = 7.6, c = 370, T = 49～60
Holotype cyst length 0.672 mm, width 0.469 mm,
Length/width 1.43
Allotype male length 1.328 mm
a = 48, b = 11, c = 330

Female and cyst: Female, white in colour first then pale yellow while living. Neck 0.07～0.10 mm in length. Cuticle 9～11 micron thick at the neck and the posterior region, 7～9 micron at the middle part of the body. Stylet 27.5 micron in length. Dorsal oesophageal gland orifice 2.5～5.5 micron posterior to the basal knobs. Vulva split, 40 micron in length. Anus located 75～90 micron anterior to the vulva. Gelatinous egg sac present and about 200 eggs in maximum extruded in it. Cyst, dark brown in colour and lemon-shaped with a prominent vulva posteriorly. Outer layer of cyst wall exhibits a rugose

pattern of zig-zag lines, and the inner layer has punctations which are arranged irregularly and appear somewhat conspicuous. Subcrystalline layer present. Brown knobs around the vulval aperture present.

Male: Head, usually with five annules, offset from the body by a slight constriction. Six lips, of which the lateral ones have slit-like amphidial openings more or less smaller in size than the others. The cephalic framework is heavily sclerotized. Four longitudinal lines are present on the lateral field. Stylet 26～27 micron (M=26.8 micron) in length with somewhat lateral to anterior protruding knobs. Dorsal oesophageal gland orifice about 4 micron posterior to the basal knobs. Spicules about 34 micron in length and bidentate type. A gubernaculum of 11.7 micron mean length present. Excretory pore located 144 ±11 micron posterior to the anterior end. Phasmids minute and located from 4 to 6 micron from the posterior end.

Type host: Soybean (*Glycine max*)

Type locality: Obihiro-shi, Hokkaido, Japan.

Type specimens: Holotype cyst (Phial No. 1) and allotype male (Slide No. 1) in collections of the Phytonemic Research Laboratory, National Institute of Agricultural Sciences, Tokyo, Japan.

Diagnosis: *Heterodera* belonging to the so-called "*schachtii* group", viz., the lemon-shaped cyst with a zig-zag cyst-wall pattern and brown knobs, as *H. schachtii*, *H. galeopsidis*, *H. avenae* and *H. trifolii*. *Heterodera glycines* most closely resembles *H. trifolii*, but differs from this species in the following respects: 1) Males of *H. glycines* are found abundant, while *H. trifolii* lacks males. 2) In the second-stage larva, distance between base of the stylet and the dorsal oesophageal gland orifice is shorter in *H. glycines* (4.0±0.2 micron) than in *H. trifolii* (7.3±0.2 micron), according to HIRSCHMANN (1956). 3) *H. glycines* matures on soybean but not on clover, whereas *H. trifolii* matures on clover but not on soybean.

Comparisons of *Heterodera glycines* with the other *Heterodera*-species The genus *Heterodera* belongs to the family Heteroderidae of the superfamily Tylenchoidea, according to THORNE (1949). The other genera which belong to Heteroderidae are *Meloidogyne*, the root-knot nematodes, and *Meloidodera* which is thought to link *Meloidogyne* and *Heterodera*.

Up to 1940, there had been a tendency to refer to all of the cyst-forming nematodes as a biological strain or race of a single species, *Heterodera schachtii* SCHMIDT, and in that year FRANKLIN (1940) raised some of these strains to the rank of a species after morphological studies of the nematodes. *Heterodera* at present contains many different species, and the identification based upon the morphological characters is exceptionally difficult. Some progress toward the identification of these species has been made in recent years, though the situation is yet far from satisfactory.

Several techniques have been offered by various workers for the identification of

Heterodera. These are based upon the measurements of the length of the larvae from cysts (FENWICK and FRANKLIN, 1951), and the characteristics of the cysts and their larvae (OOSTENBRINK and DEN OUDEN, 1954). It is, however, still difficult to identify some species of the "lemon-shaped cyst group" based upon the morphological characters of the cyst alone, and it is essential to know the cyst's host plant, colour changes of the female, the number of the eggs contained in the egg sac, the size of the eggs or larvae, and so on. OOSTENBRINK (1952) found that there are structures and features at the vulval end of some *Heterodera* cysts which make it possible to identify species or groups of species. This was further developed by COOPER (1955) who introduced some useful terminology.

The separation of *Heterodera* species is first made on the basis of the shape of the cysts. There are two groups according to whether the cyst is round or lemon-shaped. The round cysts have two very common species; *H. rostochiensis* WOLLENWEBER (the potato root nematode or the golden nematode) and *H. punctata* THORNE. The lemon-shaped cysts contain a number of species which are closely related to each other.

The separation of these lemon-shaped cysts is made first on the basis of cyst "pattern". It is said that the cyst wall is composed of three distinct layers, the outer layer is rugose and the ridges form distinct "patterns", by which two different groups can be separated. The second layer is punctate and appears as dots of a uniform size, termed "punctations", which run in some species in parallel rows, and in other species without any regular arrangement. The third layer is unmarked. The species of *Heterodera* with the cyst wall having a pattern of parallel wavy lines at right angles to the axis of the cyst, make a species group, and those which have a pattern of zig-zag lines without regular arrangement form another group. The former group is called "*cacti* group" and which has so far two described species, *H. cacti* FILIPJEV et STEKHOVEN and *H. weissi* STEINER.

The lemon-shaped cysts possessing a zig-zag lined pattern are further subdivided into two groups, according to the presence or absence of internal structures called "brown knobs". The brown knobs are located near the vulval end inside the cyst and are irregular in size, in shape, and in arrangement. They are rather conspicuous in the matured cyst, being some 10 to 20 in number. As noted before, the lemon-shaped cysts have a protuberance at the vulval end as well as at the neck. The protuberance at the vulval end was called a "vulval cone" by COOPER (1955). Within the vulval cone the brown knobs (termed "bullae" by COOPER) are found in such species as *H. major* O. SCHMIDT, *H. schachtii* A. SCHMIDT, *H. galeopsidis* FILIPJEV et STEKHOVEN, *H. trifolii* GOFFART and *H. glycines* ICHINOHE belonging to "*schachtii* group" (or "Bullata" group by COOPER).

The *schachtii* group is further subdivided into two subgroups according to the position of the brown knobs, viz., the *major* subgroup which has cysts with brown knobs situated very close to the vulva, and the *schachtii* subgroup which has the brown knobs situated a short distance within the vulval cone. The *schachtii* subgroup is composed of four species; *H. schachtii*, *H. galeopsidis*, *H. trifolii* and *H. glycines*. Thus, the separation

Many different plant species, including those susceptible and resistant to this nematode, were used for a series of experiments. They were planted mostly in pots which had been sterilized and then inoculated with cysts of *Heterodera glycines*, the cysts having been collected originally from the infested fields at Obihiro, Hokkaido. The root materials were examined with the naked eye or under a dissecting microscope. The development of larvae inside the root tissue was examined after staining the roots in hot acid fuchsin-lactophenol as recommended by GOODEY (1937). Soil temperature at different depth was recorded every day at 10 A. M. during the experiments. Examinations were made mainly on the roots at a depth of from 5 to 10 cm, in order to minimize the differences in effects of soil temperature on the development of the nematode.

3. STUDIES ON THE DEVELOPMENT OF THE NEMATODE ON PLANTS

Comparisons among host plants in the number of root-infesting females Tests were conducted in 1949 and 1950 in order to find the differences among the several susceptible plants in the degree of nematode infestation by counting the females attached to the roots. Plants were grown for about six weeks in infected pots.

Comparatively, many females were counted on soybean (*Glycine max*) and azuki bean (*Phaseolus angularis*), whereas very few females were found on kidney bean (*Phaseolus vulgaris*) and none on Spanish runner bean (*Ph. multiflorus*) and pea (*Pisum sativum*). The results are shown in Table 11.

Table 11. Comparisons among 5 plant species in the number of the root-infesting females.[1)]

Time of examination	Soybean	Azuki bean	Kidney bean	Spanish runner bean	Pea
June 1949	27.2	38.5	2.7	0	0
Aug. 1949	35.4	21.5	5.4	0	0
June 1950	38.0	39.1	2.7	0	0
Aug. 1950	14.5	7.8	1.8	0	0
Nematode-infestation index[2)]	28.3	26.7	3.2	0	0

1) Examinations based upon from 10 to 20 samples for each plant.
2) Least significant difference at 1% level: 20.4

The nematode-infestation indices derived for each of the plants tested indicate that between soybean and azuki bean no differences are found in the number of root-infesting females, whilst kidney bean is significantly unsuitable as a host of this nematode compared to soybean and azuki bean. The Spanish runner bean can not be the host plant of the nematode. The result, however, shows a discrepancy from the experiments made by FUJITA and MIURA (1934). They stated, based on the results of their experiments,

that "Among the affected plants, soybean was most severely attacked, while azuki bean was always slightly attacked. As for kidney bean and multiflora bean (= Spanish runner bean) only a trace of affection was secured."

Comparisons among soybean, azuki bean and kidney bean in the development of the larvae inside root tissue As the experiments previously mentioned show, fewer soybean cyst nematodes can mature on kidney bean than on soybean and azuki bean. A series of experiments was made in 1952 to find why these results occurred.

Two plants each of soybean, azuki bean and kidney bean grown in pots were collected every other day. The development stage of the larvae inside the roots was examined by staining, and examinations were made mainly to determine the number of days required for each life stage of the nematode to complete its development. The second-stage larva was regarded as having begun its development in the root tissue on the date of germination. The development of the adult female was taken as having terminated on the day when the embryonated egg was first observed in the egg sac attached to the female. The soil temperature at a depth of 5 cm, from June to September 1952, varied from 13°C to 28°C with an average of 20°C.

The results of this test are given in Table 12. The days required for nematode development varied a great deal according to the soil temperature but not according to the plant species tested, and it is concluded that the rates of larval development are almost the same in the three susceptible plants tested.

Table 12. Days required for the development of each life stage of the nematode inside soybean, azuki bean, and kidney bean (in days).[1]

Host plant	2nd-stage larva	3rd-stage larva	4th-stage larva	Adult female
Soybean	6～14 (8.3)[2]	3～4 (3.8)	5～6 (5.5)	11～17 (12.8)
Azuki bean	5～11 (7.5)	3～5 (4.0)	5～6 (5.8)	7～17 (11.0)
Kidney bean	6～12 (8.3)	3～4 (3.8)	5～7 (6.0)	11～17 (12.8)

1) Examinations made with four replications.
2) Averages in parantheses.

Apart from this conclusion, the author discovered why fewer females are found on kidney bean than on soybean and azuki bean. It is quite common in *Heterodera* that young adult females break the cortical tissue of roots from the inside as they develop, and they finally protrude on the surface of roots. This is true as far as *Heterodera glycines* matures on the soybean and azuki bean. In the case of kidney bean, however, young females inside the root tissue seem to be poorly developed and, in consequence, the females are small in size, producing a decreased number of eggs, and, in most cases, they hardly come out to the surface of the root. From this it is clear that very few

females are counted on the roots of the kidney bean. (Fig. 2)

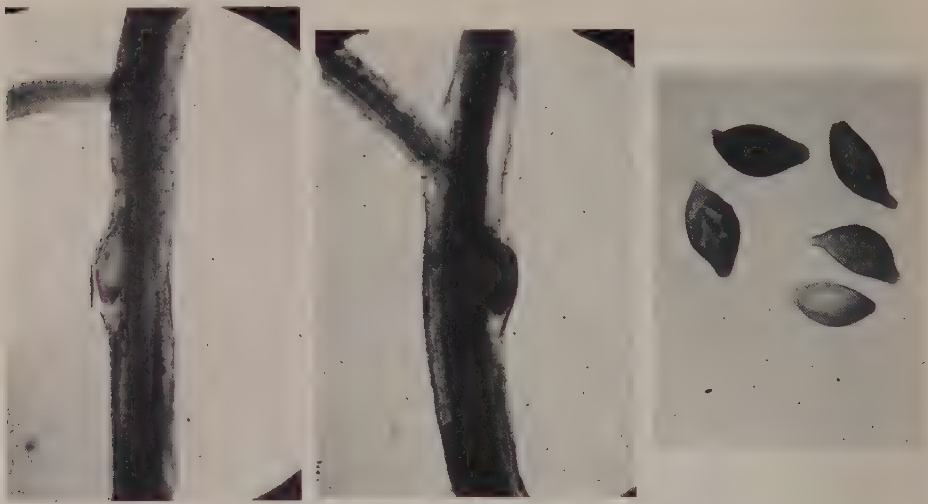

Fig. 2. The female nematodes matured on kidney bean root, indicating incomplete appearance outside root tissue and production of few eggs.

Table 13. Measurements of females matured on soybean, azuki bean, and kidney bean (in micron).[1]

Host plant	Female grown for 18 days			Female grown for 30 days		
	Length	Width	Leng./Wid.	Length	Width	Leng./Wid.
Soybean	416	211	1.97	690	478	1.44
Azuki bean	408	208	1.96	646	453	1.43
Kidney bean	414	211	1.96	453	231	1.95

1) An average of 20 females.

Table 14. Number of eggs produced by a single female matured on soybean, azuki bean, and kidney bean.[1]

Host plant	Range	Mean
Soybean	180～345	259
Azuki bean	175～356	268
Kidney bean	44～116	65

1) Based upon 10 females (=10 replications).

In August of 1952, twenty females each which had grown for 18 and 30 days in root tissue were collected from soybean, azuki bean, and kidney bean. Those collected on the 18th day were in the early adult development stage, and the females collected on the 30th day were in the stage of egg formation. Measurements of these females were made and the numbers of eggs produced were counted. The results are shown in Tables 13 and 14. The adult females which matured on kidney bean failed to develop into the same stage as those females which matured on soybean and azuki bean. It can be concluded that kidney bean is not always suitable as a host plant of the soybean cyst nematode compared with soybean and azuki bean.

Parasitism of the nematode to the non-host plants Attempts were made in 1952 to investigate further the parasitism of this nematode to various plants which have not hitherto been regarded as host plants. These included twenty-eight plant species, of which twelve species are leguminous, covering 10 families. The hitherto known host plants such as soybean, azuki bean and kidney bean were also used for comparison. They were planted in the cyst-inoculated pots, one pot per single plant. Plants were collected and stained every seventh day after their germination. Examinations were made to determine the presence of the larvae inside the roots and, if present, the development of the larvae.

The results of these tests indicated that soybean cyst nematode larvae are found to invade some plants not previously known as host plant and mostly belonging to Leguminosae. It was also found that in certain species of Leguminosae, such as Spanish runner bean, lima bean, and alsike clover, none of which had been regarded as a host plant, the nematode larvae invade root tissues occasionally and develop into partly-grown larvae. However, they do not reach adult stage.

In short the results are undernoted:

a. Twelve plant species in which no larval invasion was found.
Allium cepa (onion), *Arctium Lappa* (burdock), *Avena sativa* (oat), *Beta vulgaris* (sugar-beet), *Cucumis sativus* (cucumber), *Daucus Carota* (carrot), *Hordeum vulgare* (barley), *Hordeum vulgare nudum* (naked barley), *Linum usitatissimum* (flax), *Nicotiana rustica*, *Triticum vulgare* (wheat), *Zea Mays* (maize).

b. Seven plant species in which an occasional larval invasion was found but the larvae did not develop further.
Brassica oleracea var. *capitata* (cabbage), *Capsicum annuum* (red pepper), *Lupinus luteus* (yellow lupine), *Lycopersicon esculentum* (tomato), *Nicotiana tabacum* (tobacco), *Pisum sativum* (garden pea), *Vicia Faba* (broad bean).

c. Eight plant species in which occasionally partly-developed larvae were found but no reproduction occurred.
Medicago sativa (lucerne), *Melilotus* sp. (sweet clover), *Phseolus lunatus* (lima bean), *Pisum sativum* var. *arvense* (field pea), *Trifolium hybridum* (alsike clover), *Trifolium incarnatum* (crimson clover), *Trifolium pratense* (red clover), *Trifolium repens* (white clover).

d. One plant species in which the invaded larvae could almost mature is ***Phaseolus coccineus*** (Spanish runner bean). In the case of this plant species, the invading larvae were found to develop into fourth-stage female larvae or, occasionally, into young adult females but which fail to produce eggs. It is also observed in this plant that many male larvae can develop into the adult which later come out from the root tissues. A male larva seems to require less nourishment to mature than a female and in general the adult male appears earlier than the adult female.

4. CONSIDERATIONS ON THE POSSIBLE NUMBER OF GENERATIONS PER YEAR OF THE SOYBEAN CYST NEMATODE IN HOKKAIDO

The rate of the development of nematodes is controlled largely by their environment, namely by soil conditions and the condition of the host plant. Among these, soil temperature seems the most influential in determining the rate of the development of nematodes. It is commonly observed that the development of nematodes becomes rapid as the summer approaches and slows down towards the fall.

In order to learn the possible number of generations of the soybean cyst nematode, experiments were set up in which the length of one generation of the nematode on soybean plant in frames was examined, and repeated eight times from 1952 to 1953. Examinations were made in the following way; (1) The length of one generations, from the date of the larval invasion to the date of the emergence of the second generation's larva, was conveniently represented by the number of days from the date of germination to the date of the first recognition of the embryonated egg within the gelatinous egg sac attached to the adult female. (2) The daily soil temperature was taken at 10 A.M., it having been previously ascertained that the average daily soil temperature at a depth of 5 cm is almost the same as the 10 A.M. temperature.

The results obtained (Fig. 3) showed that the number of days required for one generation of this nematode varied from 24 to 41 days according to the soil temperature. The average soil temperature, at a depth of 5 cm, in each generation ranged from 17.8°C to 23.3°C. The relation between the rate of development, which was expressed by a reciprocal of the number of days required for one generation, and the average soil temperature during one generation was studied. The result indicated that they are roughly proportional as is shown in Fig. 4.

The author, therefore, computed an accumulated effective temperature needed to complete one generation. This was calculated as follows: As is shown in Fig. 4, the threshold temperature (t_0) is 10°C and it is the minimum temperature at which the nematode ceases to develop. The accumulated effective temperatures necessary to complete one generation were obtained by summing up the daily soil temperature which exceeded the threshold temperature (10°C) during one generation. These varied from 304 to 320 with an average of 313 day-degree. The data is given in Table 15.

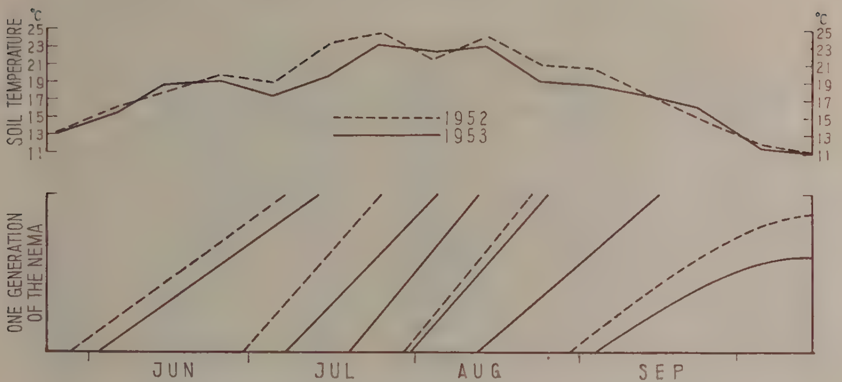

Fig. 3. Relationships between the length of one generation of the soybean cyst nematode and the soil temperature, based upon the experiments made in 1952 and 1953.

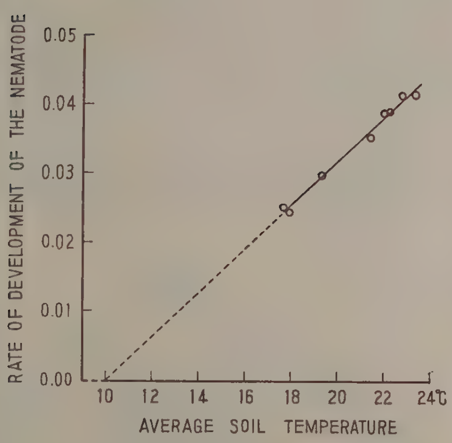

Fig. 4. Relationships between the rate of development of the nematode and the average soil temperature during one generation.

Table 15. Number of days, soil temperature, and accumulated effective temperature for one generation of the soybean cyst nematode.

Period of one generation	No. of days for one generation	Rate of development of nematode	Soil temperature at 5 cm depth during one generation (°C) Average	Range	Accumulated effective temperature for one generation (in day-degree)
3/VI ~14/VII, '53	41	0.0244	17.8	13.2~22.7	319.8
29/V ~ 8/VII, '52	40	0.0250	17.6	13.2~22.3	304.0
13/VIII~16/IX, '53	34	0.0294	19.3	14.8~23.6	316.2
8/VII ~ 5/VIII, '53	28	0.0357	21.3	17.0~26.0	316.4
31/VII ~26/VIII, '53	26	0.0385	22.0	16.5~26.5	312.0
30/VI ~26/VII, '52	26	0.0385	22.1	16.5~28.6	314.6
30/VII ~23/VIII, '52	24	0.0417	22.7	20.3~29.2	304.8
20/VII ~13/VIII, '53	24	0.0417	23.3	19.2~26.5	319.2

The following is to compute the possible number of generations of this nematode per year in Sapporo, Hokkaido. Judging from the vegetative period of soybeans in this district, the soybean cyst nematode is thought to be able to develop during the period June 1st to October 10th. The information on the soil temperature in the field during this period, from 1950 to 1953 at depths of 5, 10, 20 and 30 cm were obtained from the Hokkaido National Agricultural Experiment Station. (Fig. 5) The total effective temperature during this period was obtained by summing up the daily soil temperature which exceeded the threshold temperature (10°C). This was 1209 day-degree at a depth of 5 cm and 1069 day-degree at a depth of 30 cm. Thus, dividing the total effective temperature by the accumulated effective temperature of 313 day-degree, the possible number of generations was found as 3.8 at a depth of 5 cm and 3.4 at a depth of 30 cm. It is concluded that a maximum of three generations per year can be completed by the soybean cyst

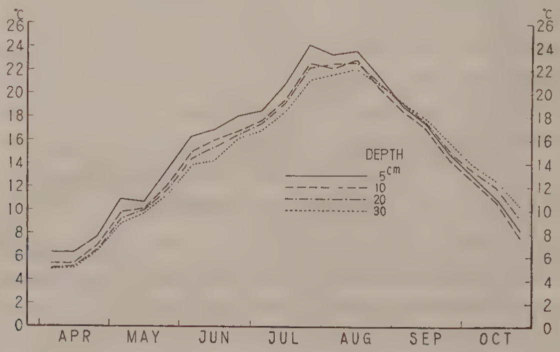

Fig. 5. Soil temperature in Sapporo, Hokkaido, during the vegetative period of plants; an average of four years from 1950 to 1953.

nematode on soybean roots, provided the other environmental conditions are favourable in addition to the soil temperatures. This data is given in Table 16.

Table 16. Possible number of generations of the soybean cyst nematode on soybean in Hokkaido.

Soil depth (cm)	Total effective temperature 1/VI～10/X (in day-degree)	Possible number of generations in maximum	The first generation	The second generation	The third generation
5	1209	3.8	1/VI～12/VII	13/VII～ 6/VIII	7/VIII～31/VIII
10	1103	3.5	1/VI～16/VII	17/VII～11/VIII	12/VIII～ 9/IX
20	1109	3.5	1/VI～18/VII	19/VII～13/VIII	14/VIII～12/IX
30	1069	3.4	1/VI～21/VII	22/VII～17/VIII	18/VIII～19/IX

5. AN EXPERIMENT ON THE NEMATODE DEVELOPMENT ON A NON-HOST PLANT GRAFTED ONTO HOST PLANT STOCK

It has not yet been known how nematodes behave on host plants when grafted onto non-host plant stocks or *vice versa*. In order to observe the case of the soybean cyst nematode, the author tried to graft host plant species on non-host plant species and *vice versa*, with some success. Grafting was made by means of "approach".*) The procedure is illustrated in Fig. 6. Soybean (*Glycine max*), *Glycine ussuriensis*, kidney bean (*Phaseolus vulgaris*), lima bean (*Ph. lunatus*) and cowpea (*Vigna sinensis*) were used for grafting. It takes seven days or so to complete one trial of grafting. The inter-genera grafting was found unsuccessful and, as is shown in Table 17, nine lima bean plant scions which were grafted on kidney bean stocks were the only successful ones in this experiment.

Table 17. Results of the inter-genera and inter-species grafting trials.[1]

Combinations of grafting		Number of trials of grafting	Number of plants successful
Scion	Stock		
Vigna sinensis	— *Glycine max*	8	0
Glycine max	— *Vigna sinensis*	28	0
Vigna sinensis	— *Glycine ussuriensis*	4	0
Glycine ussuriensis	— *Vigna sinensis*	8	0
Phaseolus lunatus	— *Phaseolus vulgaris*	16	9
Phaseolus vulgaris	— *Phaseolus lunatus*	20	0
Total number		84	9

1) Grafting made in the summer of 1954.

*) The author is greatly indebted to Mr. Masataka SAITO, Hokkaido National Agricultural Experiment Station, for instructions on grafting techniques.

Fig. 6. Procedure of grafting made in the cxperiments.

The nine successfully grafted plants were then transferred to the cyst-inoculated pots and allowed to grow for several weeks. Two of them died soon after transplanting. At the termination of growth of the plants they were collected and the development of the nematode inside the root tissues was examined by staining. The results are given in Table 18.

Table 18. Development of the soybean cyst nematode in kidney bean stocks onto which lima bean scions were grafted.

Plant	Number of days grown in infested pot	Plant growth at the time of examination of root	Measurements of females. Length×width in micron. (No. of eggs produced)[1)]
Lima bean grafted on kidney bean stock	66 (28/VII~1/X)	Plant height 22.0 cm, on flowering.	500×233 (42) 458×258 (39) 425×246 (37)
do.	51 (10/VIII~29/IX)	Plant height 31.0 cm, having a young pod. Root length 42.0 cm, with numerous root nodules.	458×285 (69) 500×258 (57) 417×200 (35) 375×217 (35) 475×225 (27)
do.	49 (12/VIII~29/IX)	Plant height 31.0 cm, having a young pod. Root length 22.0 cm.	458×267 (47) 417×233 (40) 350×208 (33) 333×167 (8) 417×233 (0)
do.	do.	Plant height 35.5 cm, having 2 young pods and a pod. Root length 31.0 cm.	475×242 (46) 492×258 (47) 492×208 (41) 392×208 (33) 533×217 (36)
do.	do.	Plant height 35.0 cm, having a young pod. Root length 38.0 cm, with many nodules.	525×250 (53) 483×250 (39) 417×204 (28) 417×175 (18) 392×192 (16)
do.	do.	Plant height 23.0 cm. on flowering. Root length 34.5 cm. Wilting of the scion remarkable.	425×208 (11) 333×175 (10) 358×167 (8)
do.	do.	Plant height 30.5 cm, on flowering. Root length 31.0 cm.	475×167 (18)

Plant	Number of days grown in infested pot	Plant growth at the time of examination of root	Measurements of females. Length × width in micron. (No. of eggs produced)[1]
Kidney bean grafted on kidney bean stock (control)	79 (13/VII∼29/IX)	Plant height 77.0 cm, having more than 10 young pods and 2 pods.	517×267 (56) 458×292 (55) 400×238 (48) 450×242 (0) 442×233 (0)
do.	51 (10/VIII∼29/IX)	Plant height 33.0 cm, having a young pod. Root length 37.0 cm.	442×233 (44) 417×225 (24) 400×192 (21) 400×158 (15) 375×117 (5)
Lima bean not grafted (control)	86 (6/VII∼29/IX)	Plant height 34.0 cm, having 7 young pods and a pod. Root length 27.0 cm.	No 4th-stage larva and adult female, but many 2nd-stage larvae, 5 3rd-stage larvae found.
do.	do.	Plant height 36.7 cm, having 9 young pods and 2 pods. Root length 29.8 cm.	No adult females, but numerous 2nd-stage larvae, 11 3rd-stage and a 4th-stage larvae found.

1) Measurements were made on the females which had attained the greatest development among the females observed.

This experiment proved that the soybean cyst nematode can mature, to some extent, on lima bean plants grafted onto kidney bean stocks, in spite of the fact that lima beans are originally non-host plant of this nematode. The adult females which had matured on lima bean plants grafted onto kidney bean stocks were evidently small in size and they produced few eggs. These females seemed very similar to those on kidney bean roots in size and in the number of eggs produced. It can therefore be concluded that the nematode can mature even on non-host plants, if these plants are grafted onto a host-plant stock; in other words, whether the invading larvae can mature or not depends upon whether the root part (not the above-ground part) is the host plant or not.

6. DISCUSSIONS ON THE PARASITISM OF *HETERODERA* SPECIES

The host plant range of nematodes is particularly important in relation to control of the disease caused by the nematodes. From the results of experiments as mentioned before, the principal hosts of the soybean cyst nematode are the plants belonging to the genus *Glycine*. Three species of *Glycine* have been reported so far to be suitable hosts, viz., *G. max* (soybean), *G. ussuriensis* (the wild soybean) and *G. gracilis*. *Phaseolus angularis* (azuki bean) seems another suitable host for this nematode. Three species of *Phaseolus*, *P. vulgaris* (kidney bean), *P. coccineus* (Spanish runner bean), *P. aureus* (mung bean), six plant species of other genera of the family Leguminosae, and *Lamium amplexicaule* (henbit deadnettle), have been recorded as host plants of this nematode, though they do not seem to be so suitable as the genus *Glycine* mentioned above.

The clover cyst nematode, *Heterodera trifolii*, has 44 different host plants covering

five families according to CHRISTIE (1959). Some of these plants are also host plants of the soybean cyst nematode. Host plants of the soybean cyst nematode and the clover cyst nematode are *Lespedeza stipulacea* and *Phaseolus vulgaris* (FRANKLIN, 1951), *Vicia villosa* (GERDEMANN and LINFORD, 1953), and *Glycine max* (SKOTLAND, WINSTEAD and SASSER, 1956). Above all, it is particularly important to note for the control of the nematodes that soybean is a common host of both nematode species. The matter is briefly discussed.

As to the parasitism of *Heterodera trifolii* to soybean plants, MANKAU and LINFORD's detailed studies (1956) have been made. They found that the larvae of *Heterodera trifolii* freely invaded the roots of 27 different varieties of soybeans, and the female larvae can reach maturity in only two varieties, "Earlyana" and "Dunford". Even in these two varieties, very few females can mature and they produce only a few eggs. They are not likely to maintain themselves for long on this plant. The workers concluded that none of the soybean varieties tested can be regarded as a suitable host of the Illinois population of the clover cyst nematode, and that it appears unlikely that any naturally occurring infestation of the clover cyst nematode will be found on soybeans.

Soybeans are reported by LIEBSCHER (1890, 1892) as a host plant of *Heterodera göttingiana*. Forty-five years after LIEBSCHER's report was published, GOFFART (1941) used the same ground and confirmed soybeans were a host plant of the same species. FRANKLIN (1951), however, states on this matter that it is possible that *H. trifolii* was formerly present on this land (i. e. in LIEBSCHER's time), since two of the species of *Vicia* which LIEBSCHER found infected are said by GOFFART to be hosts of the clover cyst nematode.

Apart from this matter, it could be agreed that there have been many discrepancies among the workers in the range of host plants of *Heterodera* species. One of the reasons for this is that many workers might use or test the combined populations or species of nematodes, due to a lack of practical methods of differentiating *Heterodera* species based on the morphological characteristics. Another reason for this, which seems more likely and more important in the author's opinion is that the nature of the parasitism of *Heterodera* species to plant species or varieties is thought to be largely quantitative rather than qualitative. It might occur that some workers think a certain plant species as the host of the nematode though it may not be accepted as a host by other workers. It is very likely to be the case that poor reproduction of nematodes occurs on certain plant species, like soybeans as a host of *Heterodera trifolii* or kidney beans as a host of *Heterodera glycines*.

V. Studies on the Population Dynamics of the Soybean Cyst Nematode

1. MATERIALS AND METHODS

The undernoted studies were made at various localities in Hokkaido where this nematode has been prevailing, and a part of the results have been reported already by

the author (1955 b, 1955 c).

The estimation of soybean cyst nematode populations in the soil was based upon the egg content or cyst content within the unit weight of soil. The plots were sampled up to a depth of 15 cm with a brass tube of 7.2 cm in diameter. From each plot five or more independent soil samples were obtained.

Extracting the nematode cysts from the soil was made by the cyst flotation method using FENWICK's can (FENWICK, 1940). A weighed quantity of dry soil was placed in the can, roiled with water, and the supernatant fluid was poured through 25 and 60 mesh sieves. This process was continued until only sand and gravel remain in the can. The residue (debris) on the 25 mesh sieve was rinsed, then discarded. The residue (containing cysts) on the 60 mesh sieve, after rinsing, was transferred to a filter paper; after excess moisture was removed it was thoroughly mixed, rolled on a board, weighed and aliquot portions were taken out on a weight basis.

The nematode population in the soil was expressed with the use of two indices: (1) number of cysts containing viable eggs and larvae per unit weight of soil, (2) number of viable eggs and larvae per unit weight of soil.

To estimate the number of viable cysts, each cyst extracted was crushed with a needle and rated through visual counts according to the egg content, and placed within a few specified grades. These gradations were set up in three ways depending upon the case, i.e. 4/4, 3/4, 2/4, 1/4, 0; 2/2, 1/2, 0; 1 and 0. Based on the number of cysts counted in each gradation, the number of the viable cysts ("fullness" cysts) was computed.

To estimate the viable eggs and larvae per unit weight of soil, the method recommended by GOODEY (1951) was employed. A hundred dry cysts taken out from a vial were soaked in water, cut into two pieces, and suspended in 100 ml. of water. The hypochlorite technique was applied to break up the egg masses and to separate eggs from the cyst wall. A subsample was withdrawn from the thoroughly mixed suspension and counting was made on eggs and larvae in 1 ml. of suspension by the use of "1 cc eelworm counting slide", with five replications. Based upon the viable eggs and larvae per cyst and the number of cysts in a weighed soil sample, the number of eggs and larvae per one gram of air-dried soil was computed.

2. FIELD EXPERIMENTS ON THE RELATIONSHIPS BETWEEN PLANT DAMAGE AND THE NEMATODE POPULATION IN THE SOIL AT HARVEST-TIME

1949 Obihiro experiment A study was made in a soybean field at Obihiro, Hokkaido in October 1949. The affected "Tokachi-nagaha" variety of soybean was sampled from seven rows, 16 to 22 plants from each row, totalling 127 plants in the field. The height, yield, and the number of new females attached to the roots were examined. Counting of the new females was made with the naked eye.

The plants varied from 30.6 to 63.9 cm in height, 6 to 76 in number of pods per plant, from 0.7 to 28.3 g seed-weight and from 28 to 226 in number of new females per

plant. The tested plants were divided into 12 gradations of growth according to the number of pods per plant, and in each gradation an average and the standard deviation were calculated. These results are shown in Table 19. Since the examination was made at harvest-time, many brown cysts which had matured on the roots examined, were thought to have detached from the roots, but it is apparent that fewer females were counted on the roots of heavily affected plants or of healthy plants than on the roots of moderately affected plants.

Table 19. Comparisons between growth of soybeans and extent of infection by the soybean cyst nematode (1949 Obihiro experiment).

Gradation No.	Number of pods	Weight of seed (g)	Height of plant (cm)	Number of females	Number of plants examined
1	8.1±1.5	1.2±0.5	43.0±3.5	53±20	9
2	13.4±1.5	3.3±1.3	40.8±5.7	66±28	7
3	18.4±1.3	3.8±1.5	45.8±5.3	80±32	24
4	22.7±1.2	4.5±1.9	45.9±4.4	94±35	19
5	28.2±1.6	6.5±0.2	48.4±7.3	87±26	14
6	33.6±0.9	7.3±2.5	49.5±3.6	140±40	9
7	38.6±1.2	10.4±2.5	50.4±4.8	135±37	8
8	41.9±0.8	11.1±1.4	52.8±6.1	142±41	8
9	47.2±1.0	12.8±3.3	51.8±3.9	166±49	5
10	52.6±1.6	13.5±2.6	53.6±4.2	165±28	5
11	56.5±0.7	16.5±3.1	52.4±3.0	102±43	8
12	66.9±2.4	20.1±3.9	54.6±3.2	118±54	11

1951 Memuro experiment A study was made in five locations at Memuro, Hokkaido in September 1951. At each location two "affected" plots and a "healthy" plot were set up within the same field based on the symptomatic conditions caused by the nematode. Fifty or more soybean plants were collected from each plot, and growth and yield of each plant were examined. The extent of the female-infestation on the roots and the nematode population in the soil in the "affected" plots were also examined. The relative index system of the root-knot nematode on plant roots introduced by SMITH and TAYLOR (1947) was employed to obtain the female-infestation indices in this experiment. Four locations out of five examined were found to be in the field planted with the "Tokachi-nagaha" variety of soybean; another one was planted to "Nakate-hadaka" variety.

Soybeans from the "affected" plots varied from 22.8 to 57.1 cm in height and 0.0 to 1.0 g in weight of seed per plant, while soybeans from the "healthy" plot were from 46.9 to 74.6 cm in height and from 5.3 to 10.2 g in weight of seed. The female-infestation indices varied from 33 to 91 per cent and the number of viable cysts per 10 g of dry soil varied from 6.0 to 14.5. These results are indicated in Table 20. No relationship between plant damage and nematode population was detected in this study.

Table 20. Comparisons between the "affected" and "healthy" plots in plant growth and the nematode population (1951 Memuro experiment).

Location No.	Height of plant (cm)		Weight of seed per plant (g)		Female-infestation index (%)	Nematode population in soil[1]
	Healthy	Affected	Healthy	Affected		
1	62.4	57.1 53.1	6.9	1.0 0.6	33 36	3.4 4.6
2	76.4	43.1 52.8	10.1	0.3 0.4	45 52	4.8 9.4
3	67.5	35.5 22.8	9.4	0.3 0.1	71 67	2.4 3.0
4[2]	46.9	33.5 25.2	5.3	0.2 0.0	50 47	4.8 5.6
5	57.5	43.5 24.2	10.2	0.3 0.5	90 91	5.8 2.4

1) Based upon the number of viable cysts in 10 g dry soil.
2) "Nakate-hadaka" variety of soybean: others are "Tokachi-nagaha".

1952 Shimamatsu experiment The study was made in September 1952, at Shimamatsu, Hokkaido in a soybean field of about one acre in extent where several yellowish patches with disease symptom appeared. Affected plants of "Manshû-daizu" variety of soybean were collected from this field, totalling 50 plants, so that they included the diverse symptomatic conditions from the heavily affected to the almost healthy. They were divided into 9 groups according to the number of pods per plant and in each group the growth and the yield of the plant were examined. Soils were sampled from the spots where the collected plants had grown, and the nematode populations were estimated.

Table 21. Relationship between plant growth and the nematode population in soybean field (1952 Shimamatsu experiment).

Number of pods	Weight of seed per plant (g)	Nematode population in soil[1]
6.3	1.3	2.6
17.8	4.4	2.9
25.8	6.6	3.5
36.3	11.8	2.8
46.4	17.7	1.8
62.0	21.9	2.1
80.8	34.8	1.2
98.2	44.9	0.6
117.5	48.1	0.2

1) Based upon the number of the viable cysts in 10 g dry soil.

The yield of individual plants varied a great deal, ranging from 6.3 to 117.5 in the number of pods per plant and from 1.3 to 48.1 g in the weight of seed per plant. The nematode populations also varied depending upon the plant growth, ranging from 0.2 to 3.5 in the number of viable cysts per 10 g of dry soil. The results are shown in Table 21. There seems to exist a high negative correlation between the yield of soybeans and the nematode populations in soil.

1953 Shimamatsu experiment The study was made in September 1953, at Shimamatsu, Hokkaido in a soybean field in which the nematode caused several diseased somewhat circular patches with yellowish and stunted plants. Examinations were made at the seven diseased patches in this field of "Manshû-daizu" variety of soybean. The undernoted five portions were selected within each diseased patch, nine or more plants and soil samples were collected from each portion, and plant growth and the nematode populations in the soil were estimated.

1) The portion situated in the center of the diseased patch.
2) The portion situated between the center and the edge of the diseased patch.
3) The portion situated inside the edge of the diseased patch.
4) The portion situated outside the edge of the diseased patch.
5) The portion situated a short distance from the diseased patch within the same field.

The plant growth and the nematode populations varied considerably with the portions within the same diseased patch. Plants varied from 20.0 to 47.3 cm in height, from 3.2 to 53.0 in number of pods, and from 3.0 to 33.0 g in weight. The nematode populations, on the contrary, decreased according to the distance from the center of the patch, ranging from 4.2 to 0.1 in the number of the viable cysts per 10 g dry soil. Apparently it can be said that the more distant the spot is from the center of the patch, the greater the growth and yield of the plant increases, and the nematode population becomes smaller. The results are shown in Table 22.

Table 22. Plant growth and the nematode population in different portions within the diseased patches (1953 Shimamatsu experiment).1)

Portion No. in the diseased patch	Height of plant (cm)	Weight of plant (g)	Number of pods per plant	Nematode population2)
1	20.0	3.0	3.2	4.2
2	30.0	9.6	15.6	2.5
3	38.6	20.0	35.9	1.0
4	41.3	28.9	56.5	0.3
5	47.3	33.0	53.0	0.1

1) An average of 7 replications.
2) Based upon the number of viable cysts in 10 g dry soil.

1954 Ebetsu experiment Attempts were made in September 1954 at Ebetsu, Hokkaido to study the relationships between plant damage and the nematode populations in a soybean field of approximately 2.5 acres, where three diseased patches appeared and which were due to the soybean cyst nematode. Fifteen "Tokachi-nagaha" soybean plants with soil surrounding were collected from each of the three diseased patches (A, B, and C). The plants and soil were also collected for comparison from outside of the diseased patch.

The results clearly indicated that the growth and the yield of plants varied remarkably within diseased patches in the same field. The plants were from 22.9 to 41.5 cm in height, from 4.0 to 20.0 g in weight of plant, and from 2.4 to 10.7 in number of pods. The nematode population also varied remarkably, ranging from 5.6 to 13.6 in the number of viable cysts per 10 g dry soil. The results are given in Table 23.

Table 23. Comparisons among three diseased patches in the same field in plant growth and yield and in the nematode populations (1954 Ebetsu experiment).

Diseased patch	Height of plant (cm)	Weight of plant (g)	Number of pods per plant	Nematode population[1]
"A"	22.9	4.0	2.4	5.6
"B"	34.2	15.6	6.2	13.6
"C"	41.5	20.0	10.7	7.0
Outside of patch	61.2	84.0	38.0	1.2 0.1 0.2

1) Based upon the number of viable cysts per 10 g dry soil.

3. EXPERIMENTS ON THE NEMATODE POPULATIONS IN CROPPING HOST AND NON-HOST PLANTS

1953 Kotoni pot experiment The experiment was conducted in 1953 at Kotoni, Hokkaido. Pots were filled with the nematode-infested soil, and soybeans of "Ishikari-shiro No. 1" variety, azuki bean, kidney bean, pea, and crimson clover were planted in each of the pots, with two replications. After cropping these plants, the nematode population in the soil was examined. Here again the population density was expressed by the number of viable cysts per 10 g dry soil.

The nematode populations were 27 and 40 for soybeans, 6 and 9 for azuki beans, 1.5 and 2 for kidney beans, 2 and 3 for peas, and 1.5 and 2 for crimson clovers. It is very clear that soybean culture results in a remarkable increase in the nematode population compared to the other tested crops.

1953 Shimamatsu field plot experiment A field plot experiment was conducted in 1953 at Shimamatsu, Hokkaido. Four plots consisting respectively of soybean ("Ishikari-

shiro No. 1" variety), kidney bean, pea and fallow were set up, with four replications. A plot was of 7.5 m^2 size. In September, after cropping these plants, soils were sampled and examined to identify the nematode populations.

The nematode population of the soybean plots was found remarkably increased, while low nematode populations were obtained from the other soil samples. The differences in nematode populations among the kidney bean, pea, and fallow plots were not clear. It is interesting to find that kidney bean seemed to decrease rather than increase the final nematode population, though this plant was thought to be the host plant of the soybean cyst nematode. The results are indicated in Table 24.

Table 24. Nematode populations[1)] of the soybean cyst nematode after cropping soybean, kidney bean, pea, and fallow (1953 Shimamatsu field plot experiment).

Plant	Replication			
	I	II	III	IV
Soybean	5.4	3.0	2.4	0.2
Kidney bean	0.8	0.2	0.4	0.2
Pea	1.6	1.6	0.6	0.0
(Fallow)	1.6	1.0	0.2	0.0

1) Based upon the number of viable cysts in 10 g dry soil.

1953 Memuro field plot experiment This is a field plot experiment made in 1953 at Memuro, Hokkaido. Soybean of "Ishikari-shiro No. 1" variety, kidney bean, pea, barley, and red clover were used to test their effects on the nematode populations. Each plot was of 6.2 m^2 size. Four replications were made. On October 19th, after cropping these plants soils were examined in order to count the viable cyst content.

The nematode population of the soybean-cropped plot had increased tremendously compared with those of the other plants. Among the kidney bean-, red clover-, and barley-

Table 25. Nematode populations[1)] of the soybean cyst nematode after cropping soybean, kidney bean, pea, red clover and barley (1953 Memuro field plot experiment).

Plant	Replication			
	I	II	III	IV
Soybean	1.6	3.7	4.5	4.0
Kidney bean	0.4	0.8	0.8	0.4
Pea	0.0	0.2	0.8	0.6
Red clover	0.4	0.8	1.6	0.6
Barley	1.4	1.0	0.6	0.8

1) Based upon the number of viable cysts in 10 g dry soil.

cropped plots, there seemed to be distinct difference in the final nematode population. The results are given in Table 25.

1954 Memuro field plot experiment This field plot experiment was conducted in 1954 at Memuro, Hokkaido. The field where the soybean disease had broken out in the previous year, was used. A nematicide "D-D" was applied in half of the field at the rate of 300 pounds per acre, on May 13th. Soils were sampled twice from both the treated and untreated plots on May 13th prior to the "D-D" treatment, and on October 19th after the harvest, to determine the initial and final nematode population levels. The following test plants were grown in the treated and untreated plots, with three replications: soybean ("Tokachi-nagaha" variety), kidney beans, Spanish runner bean, red clover, corn, and fallow plots. A plot was of 8.3 m^2 size.

The results are given in Table 26. Distribution of the nematode cysts in this field seemed rather uneven, particularly in the treated plots where the initial nematode population levels were significantly lower than those in the untreated plots. It is evident, however, that the cropping of soybean could maintain the initial nematode population level. The differences between kidney bean, Spanish runner bean, red clover, corn, and fallow in the effects from their cropping on the nematode populations are indistinct. It is again noted that the final nematode population levels are greatly decreased by cropping kidney bean which is supposed to be the host plant of this nematode.

Table 26. The initial and final nematode populations[1)] by cropping five plant species in treated and untreated plots (1954 Memuro field plot experiment).

Plant	Treated		Untreated	
	Initial	Final	Initial	Final
Soybean	1.5	1.5	4.0	4.0
Kidney bean	1.8	0.7	3.3	1.1
Spanish runner bean	1.4	0.5	3.2	0.9
Red clover	1.2	0.5	3.5	1.4
Corn	1.2	0.4	3.5	1.5
(Fallow)	1.7	0.5	2.7	1.4

1) An average of 3 replications, based upon the number of viable cysts in 10 g dry soil.

1956 Kotoni pot and frame experiments In order to study the effects on nematode population of growing host and non-host plants, experiments were conducted in 1956 at Kotoni, Hokkaido. The cyst-inoculated pots were buried in the soil up to the rim and each plant was seeded in each pot. After the plants had grown, the pots were dug out and the cysts extracted. The nematode population was estimated by the number of viable eggs and larvae per one gram of dry soil. The determination of the nematode population

was made twice; the first examination was made on July 25th while the plants were growing and the second examination was made on October 1st after the harvest.

The results of these pot experiments are given in Table 27. The transitions of the nematode population in the soil during the vegetative period of the plants were estimated based on the data obtained and as shown in Fig. 7. It was shown that the cropping of

Table 27. Effect of growing host and non-host plants on the nematode population (1956 Kotoni pot experiment).[1]

Plant	Nematode population examined on	
	July 25th	October 1st
Soybean	24	106
Kidney bean	21	31
Red clover	54	19
Pea	45	14
Potato	58	37
(Fallow)	57	39
L. S. D. (5% level)	22	30
Initial nematode population level (May 7th) = 102		

1) An average of 3 replications, based on the number of viable eggs and larvae in 1 g dry soil.

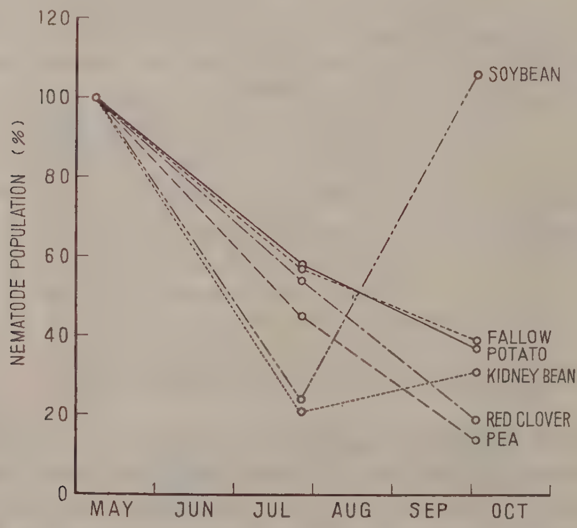

Fig. 7. Transitions of the nematode population in soil during the vegetative period of plants tested, based on the 1956 Kotoni pot experiment.

soybean evidently maintained the initial nematode population level, while kidney bean and non-host plant croppings resulted in a remarkable decrease in the final nematode population. The first examination made on July 25th showed a comparatively low nematode population in the soybean and kidney bean pots. This seems to suggest that these plants may contain or produce something which stimulates the nematode cysts to hatch early in the season.

Another experiment made by cropping plants in frames proved that growing azuki bean, one of the host plants of the soybean cyst nematode, caused a significant increase in the nematode population. The final nematode population levels in the kidney bean, Spanish runner bean, pea, and clover-cropping soils are comparatively lower than those of the other plants tested as well as in the fallowed frames. The results are indicated in Table 28.

Table 28. Effect of growing ten plant species on the nematode population (1956 Kotoni frame experiment).

Plant	Nematode population[1]		Increase ratio
	Initial	Final	
Azuki bean	77	216	2.80
Soybean	59	66	1.12
Kidney bean	70	25	0.36
Spanish runner bean	59	25	0.43
Pea	76	36	0.47
Red clover	73	35	0.48
Broad bean	83	46	0.56
Lima bean	82	46	0.56
Upland rice	67	44	0.65
Potato	73	53	0.73
(Fallow)	66	41	0.63

1) An average of 3 replications, based on the number of viable eggs and larvae in 1 g dry soil.

1956 Shimizu field plot experiment The experiment was made in 1956 at Shimizu, Hokkaido. Six kinds of plants were grown in each plot, with three replications. A plot was $4\,m \times 2.2\,m$ in size.

The decrease in final nematode population was remarkable in the plots of three kidney bean varieties compared with those of the fallowed or potato plots. A moderate decrease was also found in the red clover-cropped plot. Due to the unfavourable climatic conditions in that year plant growth was generally very poor. The soybean-crop failed to maintain the initial nematode population level, probably because of the extremely poor growth of the plant itself. The results are given in Table 29.

Table 29. Effect of growing plants and fallowing on the nematode population (1956 Shimizu field plot experiment).

Plant	Nematode population[1)]		Increase ratio
	Initial	Final	
Kidney bean (A)[2)]	116	24	0.20
Kidney bean (B)	91	26	0.28
Kidney bean (C)	106	30	0.28
Red clover	107	46	0.43
Soybean	106	49	0.47
Potato	93	55	0.59
(Fallow)	85	49	0.58

1) An average of 3 replications, based on the number of viable eggs and larvae in 1 g dry soil.
2) The names of the varieties of kidney bean are: (A) Ôtebo, (B) Beni-kintoki, (C) Chû-naga-uzura.

Table 30. Effect of growing host and non-host plants on the nematode population (1957 Kotoni pot experiment).

Plant	Final nematode population[1)]		
	Exp. I	Exp. II	Exp. III
Soybean	92	12	—
Azuki bean	—	19	—
Kidney bean	17	—	5
Red clover	10	—	19
Broad bean	6	6	—
Lucerne	13	—	16
Pea	—	7	—
Lima bean	—	4	—
Lespedeza sp.	—	42	—
Potato	12	—	14
Rape	17	—	27
Artemisia sp.	—	7	—
Mint	—	15	—
Flax	—	16	—
Buckwheat	—	10	—
Barley	—	23	—
Rye	—	18	—
Corn	13	—	—
Oat	—	—	26
(Fallow)	55	18	26
L. S. D. (5% level)	20	8	14
Initial nematode population	57	88	88
Date of examination	30/X	21/VIII	16/IX

1) An average of 4 replications.

1957 Kotoni pot experiment A series of pot experiments was conducted in 1957 at Kotoni, Hokkaido. Nematode populations were expressed by the number of viable eggs and larvae in 1 g dry soil.

The results of the experiments are given in Table 30. These results, except for "Experiment II" generally agree with the results already mentioned. In "Experiment II", the soybean crop again failed to maintain the initial nematode population level. This probably comes from the fact that the determination of the final nematode population was made earlier than those in the other experiments.

4. EXPERIMENTS ON THE NEMATODE POPULATIONS IN A SUCCESSIVE SOYBEAN-CROPPED PLOT

Experiments were made during the years 1955 to 1957 at one of the successive cropped plots in the Hokkaido National Agricultural Experiment Station. Since 1927, there has been practiced at this station successive croppings of various kinds of crops for experimental purposes. In July 1955, soybean plants in the successive soybean-cropped plot indicated symptoms caused by the soybean cyst nematode for the first time. The plot was 3 m wide and 2 m long. Six rows, except in 1956 when seven rows were mistakenly used, were set up from south to north, and 13 soybeans of "Ooyachi" variety were planted in each row. Each row was conveniently divided into three "subplots", each of which included 4, 4, and 5 plants respectively from the south to the north, and thus 18 subplots (21 subplots in 1956) in all were set up. The plant growth, yield and nematode population in the soil were observed in each subplot at every harvest. Nematode populations were expressed by the number of cysts, regardless of their egg content, per 10 g of dry soil and the data obtained over the three years from 1955 were statistically analyzed. Fertilizing, seeding and other cultural practices were made in the routine manner throughout the experimental period.

Relationships between the nematode population and the soybean growth in the plot The growth and the yields of soybeans and the nematode population obtained are

Table 31. The growth and yields of soybean and the nematode populations in the successive soybean-cropped plot.[1)]

Year	Height of plant (cm)	Number of pods per plant	Number of seed per plant	Weight of plant (g)	Weight of seed per plant (g)	Nematode population[2)]
1955	51.4	27.8	49.6	34.4	15.2	7.5
1956	45.8	21.2	31.8	18.0	8.1	11.6
1957	41.5	25.0	36.8	18.6	8.8	27.4

1) An average of 18 subplots (replications) and 21 for 1956.

2) Based upon the number of cyst in 10 g dry soil.

shown in Table 31. It is evident that the successive cropping of soybean caused a remarkable decrease in the growth and yields of plants and increased the nematode population. Since the climatic conditions in 1956 were so unfavourable that most crops in Hokkaido suffered badly from cold damage, due to unusual low temperatures during the summer, the growth and yield of soybeans in that year showed a considerable decrease.

From the results obtained, the relationships between the nematode population in soil and the plant growth and yield were studied. The first move then was to study to what extent the plant characters, such as height of plant, weight of seed and so on, are correlated with the nematode population in the soil.

Coefficients of correlation between the nematode populations and each of the plant characters were computed. They are:

height of plant	$r = -0.665^{**}$
weight of plant	$r = -0.654^{**}$
weight of seed	$r = -0.640^{**}$
number of seed	$r = -0.579^{**}$
number of pods	$r = -0.534^{**}$

where r= correlation coefficient between the character indicated and the nematode population, ** $P<0.01$, and $n=57$. In particular the height and weight of the plant and the weight of seed per plant were most closely correlated with the nematode population at harvest-time.

Since the weight of seed is the most important of these plant characters, the relationship between the nematode population and the weight of seed per plant was further studied by computing the coefficient of regression (b). The resulting calculation showed

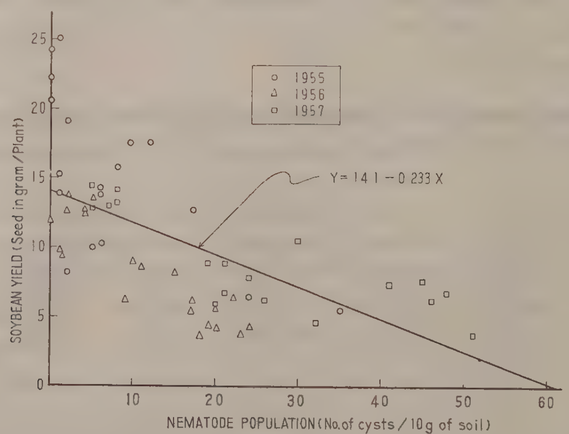

Fig. 8. Relationship between the nematode population and the soybean yield, based on the experiments in the successive soybean-cropped plot during the years 1955 to 1957.

that b = −0.233 (g). An equation of regression is shown in Fig. 8. The equation of regression obtained means as follows:

Provided the plot is free from the nematode cysts, viz., the nematode population is zero, plants will produce 14.1 g of seed per plant. On the contrary, provided the nematode population increases up to 60.5 by the number of cysts per 10 g of soil, plants will produce no seed.

Annual transitions of plant symptoms, nematode populations and soybean yields The general feature of the annual transitions of plant symptoms and the nematode population in the plot experimented with during the years 1955 to 1957, can be conceived by Fig. 9. In this figure, "subplots" where soybeans indicated the yellowish symptoms caused by the nematode are shaded with oblique lines. As is shown in Fig. 9, the plant symptoms appeared in only two subplots out of 18 in 1955, extending to 13 subplots out of 21 in 1956, and finally covering 13 subplots out of 18 in the third year from the initial outbreak of symptoms. According to the observations made on July 12th, 1955, the plant symptoms of stunting and yellowing were restricted to only two subplots, though the adjacent plots afterwards showed very slight yellowing on August 6th. According to the observations made on July 9th and August 11th, 1956, the most remarkable plant symptoms appeared in two rows situated in the extreme west, though the adjacent two rows indicated only moderately yellowish and stunted symptoms. In 1957, almost all subplots indicated the typical plant symptoms throughout the growing season. The annual transitions of the nematode populations in the soil are very remarkable and they show a striking increase. In the third year from the initial outbreak of the symptoms, the soil in all subplots was found to contain nematode cysts.

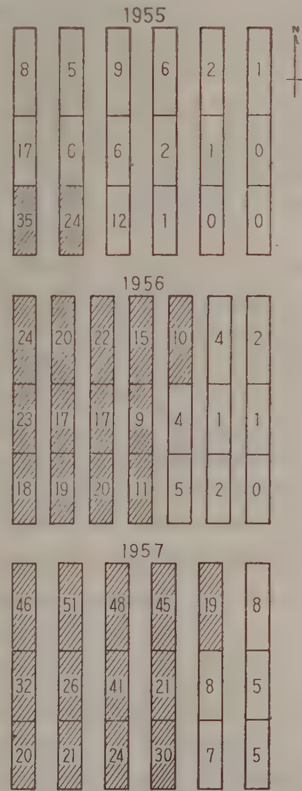

Fig. 9. Annual transitions of the plant symptoms and the nematode populations in the successive soybean-cropped plot during the years 1955 to 1957; the yellowish discoloration of plants caused by the nematode is shaded with oblique lines, and figures within the subplots indicate the number of nematode cysts per 10 g dry soil.

VI. Studies on the Resistance of Soybean Varieties to the Soybean Cyst Nematode

1. HISTORICAL REVIEW

It has been well known that the resistance of soybean plants to the soybean cyst

nematode differs according to the variety, but a variety with strong resistance has rarely been found, though many attempts have been made in Japan to find such highly resistant varieties. Since about 1945 it has been said that a few varieties which are highly resistant to the disease exist in the northern part of Honshu (mainland of Japan). These varieties are "Daiichi-hienuki", "Nangun-takedate", and "Kurosaya-sanbongi". They can mature only during the last part of October, even in their original localities, though they cannot mature in Hokkaido. No detailed study has been made on the nature of the varietal resistance of soybean.

ICHINOHE and ASAI (1956) studied the resistance of the soybean varieties "Daiichi-hienuki" and "Nangun-takedate" to this nematode, and they found that the undernoted ecological features of these varieties seemed to be associated with their resistance, viz., (1) failure of any large number of the nematode larvae to survive after invasion, (2) increase in the activity of root-nodule bacteria, (3) strong root growth and, in consequence, a good health of the entire plant.

ROSS and BRIM (1957) reported several soybean varieties which seem highly resistant to this nematode. These varieties are "Ilsoy", "Peking", "P. I. 90763" and "P. I. 84751"

ROSS (1958) made histological studies on the resistant soybean varieties and found that in the roots of the resistant plants no giant cells are observed and that the reaction of this resistant variety to the nematode indicates a type of hypersensitivity.

2. INVESTIGATIONS ON THE NATURE OF RESISTANCE OF PLANTS TO NEMATODES

Works on the nature of the resistance of plants to nematodes, which have been made mainly from the cytological aspects of plants, are described below.

The report of the root-knot nematode by BARRONS (1939) is the first and important information on the nature of resistance of plants to nematodes. The report includes two major points of interest, the first being that the degree of nematode invasion in the roots is the same in all cases regardless of whether the host is susceptible or not to the nematode. The second important point is that the plant reacts to the secretion of the nematode by forming certain compounds which stimulate the formation of giant cells in the root tissue. These two points are understood to be the origin of resistance to the root-knot nematode. CHRISTIE (1949), however, showed that many root-knot nematode larvae invaded alfalfa while very few invaded lantana (a plant of the genus *Verbena*). DONCASTER (1953) also reported the same fact with a species of *Heterodera*. Therefore, BARRONS' theory, that the resistance does not depend on the number of larvae which invade the root, is not necessarily correct. However, this raises important questions on the nature of the parasitism of nematodes. DROPKIN (1954) reported that the degree of the nematode invasion is explicitly different between tomato and cucumber, both of which are invaded by the root-knot nematode, *Meloidogyne incognita.*

It is noted that after the invasion of nematodes giant cells are usually produced in the plant by the stimulus of the nematode. In the case of resistant plants, giant cells are

ot produced and the breakdown of cells occurs. CHRISTIE (1949) reported that highly esistant plants surround the invading larvae with extremely vacuolated cells so that the ırvae starve to death since they cannot obtain nutrition. Furthermore, he reported that ıany broken cells are found in the root of the resistant plants near the invaded larvae, ɔ that the nematode occasionally may not be able to grow after invasion. KÔSAKA ormerly MIZOGAMI) (1947) confirmed that the resistance of sweet potatoes to the root-not nematode has a close connection with whether or not giant cells were formed after ıe invasion of the nematode, and that the cells around the invading larvae are poisoned nd turn yellow and giant cells were not produced; even if giant cells are produced they ɔon die and the nematodes also die in due course.

SHIBUYA (1952) clarified that the larvae of the root-knot nematode (probably *Meɔidogyne incognita acrita*) invade both resistant and susceptible varieties of sweet potatoes ɔ the same degree. He considered the resistant varieties do not allow the invading ırvae to develop due to some chemical and physiological factors, not because of any ıorphological factors of the root. He reported that the reason why the resistant variety oes not usually form giant cells will be clarified by comparing the protein from resistant ith that of the susceptible varieties by means of immuno-chemical methods, since the rotein from the former probably has many special characters which are not exhibited y protein reactions in the latter.

CRITTENDEN (1954) reported that the resistance of soybean to the root-knot nematode *Meloidogyne incognita acrita*) is due to the following morphological and physiological ıaracters of the soybean plant. (1) The root grows straight and deeply into the soil and as only a few lateral roots. (2) The root is soon lignified. (3) It can grow well even in oil which contains a small amount of potassium. (4) Only a small amount of fat is resent in the beans of the resistant varieties.

KÔSAKA (1950) considered that the resistance of sweet potato to the root lesion ematode, *Pratylenchus* sp., appears after the invasion of the larvae and this is the ıaracteristic nature of resistant varieties, since there is no difference in the disease pattern the beginning of the nematode invasion between resistant varieties and susceptible arieties. In the resistant varieties, the nematode cannot move into new parts because corky layer is soon formed and it therefore dies. This layer separates the diseased ssue from the healthy tissue and the cell-poisoning occurs very early, furthermore, he eported that the formation of a wound periderm in slender roots is considered to have relation to some secretion from the nematode.

There are almost no reports concerning resistance to the genus *Heterodera*, except ɔr *H. rostochiensis* which is a very important parasite of potatoes in Europe. The potato ɔot was found to secrete some matter which expedites the hatching of the larvae from ıe cyst. JONES (1954) stated that two plant species, *Solanum ballsii* (*S. vernei*) and *S. ındigenum*, were found resistant to this nematode by ELLENBY in 1941. Since then esearch has been made to utilize these two species in a breeding programme. JONES proved ıat the two species and their lines are the same as the other susceptible lines so far as

the larval invasion of the root is concerned, though no larvae can develop into adult females in these two plants. He also stated that the secretion of these plants expedites the hatching of the larvae from the cysts just like other susceptible varieties. On the other hand, DONCASTER (1953) clarified, by comparing the parasitism of *H. rostochiensis* on tomato and *Solanum nigrum*, that the number of larvae invading per unit weight of root of tomato is always greater than that of *Solanum nigrum*, and he also found a clear difference in the number of invading larvae between these two plants. The destruction of the root tissues of *Solanum nigrum* was found at the point of larval invasion, therefore, the larvae died and the number of adults, especially females, was lessened. This was not found in the roots of tomato.

3. MATERIALS AND METHODS

Studies on the varietal resistance to the soybean cyst nematode described below were made in 1955 at the Hokkaido National Agricultural Experiment Station. The result was reported previously by the author and ASAI (1956).

Plant materials, including the resistant and susceptible varieties were obtained from this station or from the original supplying experiment stations. The varieties were grown in pots or frames to which the nematode cysts were inoculated. The inoculum used in the pot experiment of this study was obtained by floating and screening the infested soil through 20 and 70 mesh sieves. This cyst mixture contained many cysts, soil particles, seeds, small fragments of roots, and other organic matter. This mixture was dried, mixed thoroughly and a weighed portion put into pots. For control, no cyst mixture was added though the pots were prepared under the same conditions of seeding, soil, climate, and disposition.

The characteristics of the soybean varieties and plants used in the study were as follows: (1) "Daiichi-hienuki", a variety from Akita Prefecture and now bred at Kariwano Branch of Tohoku National Agricultural Experiment Station. The history of this variety is obscure though it has been recognized to be one of the varieties most resistant to the soybean cyst nematode. (2) "Nangun-takedate", a variety indigenous to Aomori Prefecture, and was exhibited in the soybean competition of 1945. This is regarded as a comparatively resistant variety to the soybean disease caused by the nematode. (3) "Tokachi-nagaha", a variety arising from an artificial cross, and very commonly cultivated throughout Hokkaido. This variety is a late-medium to early-late maturing soybean, and a susceptible variety to the soybean cyst nematode. (4) "Kokusô", an extremely early variety produced in Manchuria. Though it has been considered from early work more resistant than "Tokachi-nagaha", it is in fact a rather susceptible variety. (5) *Glycine gracilis*. This is a plant species very closely related to soybean and the seeds were originally obtained from Illinois, U. S. A. The seed is light brown in colour and it is smaller than the smallest soybean. The susceptibility to the soybean cyst nematode has not yet been clarified.

4. COMPARISONS BETWEEN THE RESISTANT AND SUSCEPTIBLE VARIETIES IN THE GROWTH AND YIELD OF SOYBEAN

Comparisons under normal environment "Daiichi-hienuki" and the other four varieties were planted on May 21st in pots to which the cyst mixture was previously inoculated, so that the nematode population in the upper 15 cm layer of soil was 26.5 viable eggs per 1 g of soil. Two replications were made and all the test pots were kept in a greenhouse. Although "Daiichi-hienuki" and "Nangun-takedate" had not matured in the writer's past tests, they reached maturity in this experiment under the greenhouse conditions.

The yellowish plant symptoms were clearly recognized on "Tokachi-nagaha", "Kokusô", and *G. gracilis* in the infested pots during the last ten days of July, while only slightly yellowish symptoms were recognized on "Nangun-takedate" and no symptoms on "Daiichi-hienuki".

The height and weight of the plants at harvest-time are given in Table 32. Neither "Daiichi-hienuki" nor "Nangun-takedate" had suffered much damage as compared with the other varieties. Especially the former was superior. Plant symptoms and the damage by this nematode to *G. gracilis* are evident and this plant gives indications of being highly susceptible.

Table 32. Height and weight of soybean varieties grown in pots inoculated at the same levels with *H. glycines* cysts and uninoculated control.[1]

Variety	Height of plant (cm)		Gross weight (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Daiichi-hienuki	119.8	114.4 (96)[2]	68.7	80.2 (105)
Nangun-takedate	86.5	73.6 (85)	69.8	66.3 (95)
G. gracilis	93.6	66.6 (71)	27.5	16.8 (61)
Kokusô	38.5	38.0 (99)	29.3	16.5 (56)
Tokachi-nagaha	48.6	40.4 (83)	60.5	25.7 (42)

1) An average of 2 replications.
2) Per cent ratio against the "uninoculated" put in parentheses.

Comparisons under short-day treatment Experiments were made with three varieties, "Daiich-hienuki", "Nangun-takedate" and "Tokachi-nagaha", grown in pots likewise prepared under short-day treatment. The pots were kept in a greenhouse after they emerged on May 28th. They had natural light from 9 A.M. to 5 P.M. and then the pots and plants were covered with black cloth during the rest of the time. This treatment was continued from June 6th until the end of the flowering period on July 8th.

All varieties used flowered early and the growing days were greatly shortened as a result of the short-day treatment. It was also apparent that the plants were stunted and the yields were very small. "Daiichi-hienuki" and "Nangun-takedate" flowered on July 5th and 6th, and matured on September 12th and 13th respectively. "Tokachi-nagaha" indicated typical disease symptoms in the inoculated pots and gave a remarkable loss of gross weight and weight of seeds. Slight damage to "Nangun-takedate" was recognized though "Daiichi-hienuki" grew better in the inoculated pot than in the control. The results are indicated in Table 33.

Table 33. Height and yield of soybean varieties under short-day treatment from June 6th to July 8th, 1955.[1]

Variety	Height of plant (cm)		Gross weight (g)		Weight of seed (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
Daiichi-hienuki	27.6	30.3 (110)	17.0	19.9 (117)	10.3	11.0 (106)
Nangun-takedate	35.4	33.6 (95)	26.6	23.8 (90)	13.7	12.6 (92)
Tokachi-nagaha	27.6	24.5 (89)	12.6	8.6 (68)	7.5	5.1 (68)

1) An average of 2 replications and per cent ratio put in parentheses.

Comparisons under long-day treatment Other experiments were made with "Tokachi-nagaha" and "Kokusô" under long-day treatment. They were planted on June 15th in pots prepared as in the previous experiments. Artificial illumination at night was given in the greenhouse from a 60-W bulb at a distance of 1 meter from the plants. This light was in addition to normal daylight and it was available from June 25th until the end of the flowering period on August 15th.

The effect of this treatment was to greatly delay flowering and to prolong maturation respectively to August 11th and November 4th for "Tokachi-nagaha". The variety

Table 34. Height and yield of the soybean varieties under long-day treatment from June 25th to August 15th, 1955.[1]

Variety	Height of plant (cm)		Gross weight (g)		Weight of seed (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
Kokusô	129.5	132.9 (103)[2]	13.9	11.8 (85)	6.3	4.3 (68)
Tokachi-nagaha	122.6	118.8 (97)	18.8	13.8 (73)	6.5	3.7 (56)

1) An average of 2 replications.

2) Per cent ratio put in parentheses.

"Kokusô" was not so delayed, the dates of flowering and maturation being July 29th and September 21st respectively. Since the vegetative growth of the two varieties was abnormally increased due to the effect of the long-day treatment, the yield was very small. The plant symptoms were not clear although the damage to both varieties in the inoculated pots was remarkable. The results of this test are given in Table 34.

Nematode populations after growth of soybean varieties in pots Examinations of nematode populations in the pots were made following the growth of resistant and susceptible varieties. The weight of soil to a depth of 15 cm in the pot was 7,110 g.

Table 35 shows the final nematode population levels expressed by the number of eggs per one gram of soil, and the increase ratio of the final nematode population level against the initial nematode population level. Since the number of days varied for which the plants were allowed to grow in the pots, according to the maturing time of the variety tested and the treatment undergone, no clear trend could be seen from Table 35 concerning the effect from different growing conditions and varieties on the nematode population. "Daiichi-hienuki", however, indicated a comparatively low level of final nematode population, notwithstanding that this variety had been grown in pots over the longest period among the varieties tested.

Table 35. Final nematode population levels in upper 15 cm layer of soil where the resistant and susceptible varieties were grown during different periods.[1)]

Variety	Number of days for which plant grew	Number of cysts per 1 g soil	Number of eggs per 1 g soil	Increase ratio[2)]
Group I (normal environment)				
Daiichi-hienuki	159	1.5	50	1.9×
Nangun-takedate	150	2.0	104	3.9×
G. gracilis	107	3.8	149	5.6×
Kokusô	98	2.3	43	1.6×
Tokachi-nagaha	117	4.3	178	6.7×
Group II (short-day treatment)				
Daiichi-hienuki	107	2.0	37	1.4×
Nangun-takedate	108	2.3	84	3.2×
Tokachi-nagaha	93	2.4	41	1.6×

1) An average of 2 replications.
2) Initial nematode population = 26.5 eggs per 1 g soil.

5. COMPARISONS OF THE NEMATODE BEHAVIOR INSIDE THE ROOT TISSUES BETWEEN RESISTANT AND SUSCEPTIBLE VARIETIES

A cyst mixture of 200 mg (average number of cysts 1,700; 23 eggs per cyst) was evenly

mixed into the soil to a depth of 5 cm. One kg of steam sterilized soil was put in each unglazed pot of 15 cm in diameter. These pots were buried into the soil up to the rim and "Daiichi-hienuki", "Nangun-takedate", "Kokusô" and "Tokachi-nagaha" varieties were planted on May 28th. Nine pots were used for each variety. Each pot was thinned to 3 plants on June 10th, and to 2 plants after June 22nd. Roots were removed from three pots of each variety on the 3rd, 14th, and 30th days after the date of emergence, these roots being washed carefully. Two, three, or four roots from each pot in the first test and one root from each pot in the second and third tests were sampled to examine the number of larvae that had invaded the roots. The weighed roots were stained with acid fuchsin-lactophenol solution. In the third test, the roots were cut into small fragments 1 cm in length and the number of larvae found was counted.

Number of larvae present in the roots Since the roots in the first test were very small as they were sampled shortly after the date of emergence, there were very few larvae in the roots. It was not made clear from the results indicated in Table 36 if the number of invaded larvae differs according to the varietes tested.

Table 36. Number of larvae invading the roots of resistant or susceptible varieties on the 3rd day from the date of emergence (10/VI, 1955).

Variety	Number of roots examined	Total weight of root (g)	Number of larvae invaded	Number of larvae per 1 g root
Daiichi-hienuki	11	4.3	0	0
Nangun-takedate	9	3.2	9	3
Kokusô	8	2.7	3	1
Tokachi-nagaha	8	3.4	6	2

Many of the invading larvae were recognized in the roots in the second test on the 14th day from the date of emergence. Almost all the larvae that entered the roots were of the second-stage, and there were few third-stage larvae. The number of larvae per 1 g of root of each variety did not indicate a significant difference between varieties, since

Table 37. Number of larvae invading the roots of resistant or susceptible varieties on the 14th day from the date of emergence (21/VI, 1955).[1]

Variety	Weight of root (g)	Number of larvae invaded	Number of larvae per 1 g root
Daiichi-hienuki	2.0	116	57
Nangun-takedate	1.9	101	52
Kokusô	1.9	71	37
Tokachi-nagaha	2.1	92	44

1) An average of 3 replications.

there were great differences within the same variety. The average number of larvae in 1 g of root was 52 ± 42 ($n = 12$). The result of the test is given in Table 37.

The number of larvae in the roots increased remarkably in the third test on the 30th day from the date of emergence. Many third-stage and fourth-stage larvae, as well as the second-stage larvae, were found and the adult males were recognized in this case. Although the number of larvae per one gram of root increased enormously since the preceding test, no significant differences between varieties were obtained, because of the great differences evident within the same variety. An average number of larvae per one gram of root was 431 ± 145 ($n = 12$). The results given in Table 38.

Table 38. Number of larvae invading the roots of resistant or susceptible varieties on the 30th day from the date of emergence (7/VII, 1955).[1]

Variety	Weight of root (g)	Number of larvae invaded	Number of larvae per 1 g root
Daiichi-hienuki	3.5	1,710	493
Nangun-takedate	4.4	2,377	544
Kokusô	4.1	1,272	313
Tokachi-nagaha	4.5	1,571	349

1) An average of 3 replications.

Dead larvae after invasion Examination of the soybean roots on the 30th day from the date of emergence, revealed that there were some larvae whose intestines were almost vacant and unstained. The cuticle of these larvae was shrunken. The death of the larvae would be caused by something after the invasion. Some varieties had many of these larvae. Most of the larvae were second-stage, however, some dead larvae were recognized as third-stage or even fourth-stage, though they were very rare. Fig. 10 shows the appearance of these larvae.

There was a distinct difference among varieties in respect of the number of dead larvae. Comparatively they were numerous in "Daiichi-hienuki" and "Nangun-takedate" and few in "Kokusô" and "Tokachi-nagaha". Thus, the number of dead larvae appeared to correspond to some extent with the degree of resistance of the variety. Sometimes it was difficult to distinguish the dead larvae from healthy ones at a quick glance under the dissecting microscope. However, the differences in the number of dead larvae between varieties can be shown by comparing the total number of second-stage larvae found inside the root tissue, since most of the dead larvae are at this stage and the healthy ones soon develop into third-stage larvae. Table 39 shows the percentage of the number of each larval stage counted in the root of each variety on the 30th day from the date of emergence. In "Daiichi-hienuki", 87 per cent of the larvae found inside the root tissue were in second-stage, and this variety had the largest number of dead larvae compared to all other tested varieties.

(a) (b) (c)

Fig. 10. Microphotographs of the dead larvae of soybean cyst nematode in the root tissue of soybeans. (a) and (b) Dead second-stage larva. (c) Dead third-stage larva.

Table 39. The percentage of the number of each larval stage counted in the roots of resistant and susceptible varieties on the 30th day from the date of emergence.[1]

Varieties	2nd-stage larva	3rd-stage larva	4th-stage larva
Daiichi-hienuki	87	11	2
Nangun-takedate	73	20	7
Kokusô	64	26	10
Tokachi-nagaha	51	32	17

1) An average of 3 replications.

Number of matured females An experiment was made by replanting nematode-infested soybean seedlings into sterilized soil to allow the larvae to develop further. The plants used in the preceding experiment were employed. Two plants from each pot on the 14th day after the emergence and one plant from each pot on the 30th day after the emergence, were sampled. These were replanted after washing the roots of one or two

plants into each of a series of pots previously filled with sterilized soil. The plants were then placed in a greenhouse and the soil temperature was recorded at 10 A.M. each day. The number of adult females matured on the soybeans in the pots was examined by extracting the females from the soil by FENWICK and REID's technique. The above procedure was carried out on the plants which had been transplanted on the 14th and 30th days from the date of emergence, on July 25th and August 3rd respectively. The dates of the examination were determined by summing up the accumulated effective temperatures for the development of the larvae during the period from the date of replanting until the date of the examination in question, so that the computed accumulated effective temperatures were greater than the accumulated effective temperatures needed for one generation of the nematode, 313 day-degree, but less than those needed for two generations.

On the soybean plants replanted on the 30th day from emergence, there were few matured females in "Daiichi-hienuki" and "Nangun-takedate" compared to the other susceptible varieties. The differences between these two groups in the number of matured females were very clear and as shown in Tables 40 and 41.

Table 40. Number of matured females, per 2 plants, on soybean varieties replanted into sterilized soil on the 14th day from the date of emergence. (Examined 25/VII, 1955)

Variety	I	II	III	Total
Daiichi-hienuki	1	1	—[1]	2
Nangun-takedate	9	7	1	17
Kokusô	22	14	13	49
Tokachi-nagaha	9	8	7	24

1) Discarded due to error of test.

Table 41. Number of matured females, per one plant, on soybean varieties replanted into sterilized soil on the 30th day from the date of emergence. (Examined 3/VIII, 1955)

Variety	I	II	III	Total
Daiichi-hienuki	13	10	3	26
Nangun-takedate	8	5	1	14
Kokusô	96	40	31	167
Tokachi-nagaha	78	54	45	177

There were very few matured females in all varieties compared with the number of larvae entering the roots, as indicated in the preceding experiment. The ratio of matured females to invading larvae, counted in the preceding experiment, was estimated to be about 4 per cent in "Tokachi-nagaha" and "Kokusô", and less than 1 per cent in "Nangun-

takedate" and "Daiichi-hienuki". The reasons for this were as follows; (a) The invading larvae died from injury caused to the root by the replanting procedure. (b) FENWICK and REID's technique cannot extract all of the adult females out of the soil. These two reasons are thought to be experimental errors. (c) The larvae die after the invasion for some reasons regardless of replanting. (d) The numbers of male larvae and adults were neglected. Even so, it was evident from this experiment that "Daiichi-hienuki" and "Nangun-takedate" contained only a few matured females compared to the susceptible varieties. This fact must be due to (c) to a great extent.

6. COMPARISONS OF THE MORPHOLOGICAL AND ECOLOGICAL CHARACTERS OF THE ROOTS

"Daiichi-hienuki" and three other varieties as previously included were planted in frames of 1 square meter size, one plant to each frame, in which an approximate equal weight of infested soil was placed and well-mixed. The frames used as the control were sterilized with "D-D" in the fall of 1953.

Seeding was made on May 21st, emergence occurred on June 4th, and each plant was thinned to bear two stems. The first test was done on August 7th~8th, and the second on September 6th~9th. Each test was replicated twice. The root was dug out as deeply as possible, soaked at once in 5% formalin for 30 minutes, washed, dried and weighed. The number of laterals with a diameter of more than 2~3 mm was counted. The number of root-nodules were counted with the naked eye and the numbers removed due to the washing were added, these latter being obtained by screening. The adult females on the surface of the root were removed with a brush and caught on a 70 mesh sieve. The adult females which had separated from the root and dropped into the soil at the bottom of the formalin solution were collected by the method of FENWICK and REID (1951), with a saturated solution of zinc sulfate. The total number of these adult females were determined with FENWICK's counting dish after diluting.

The nematode populations in the control frames were investigated in order to confirm the effect of the "D-D" fumigation. The number of invading larvae on "Tokachi-nagaha", which was planted on May 21st and emerged June 3rd was 947 (68 per 1 g of root) in the non-treated frames, and 0 in the treated frames. The nematode count on June 30th was 1,096 (42 per 1 g of root) and 5 (0.2 per 1 g of root) respectively. The above data was calculated from the average of four plants.

Weight of roots and number of lateral roots No differences in the weight of roots between the infested areas and the control areas could be recognized, although the differences among varieties were clear. There was a notable increase in the weight of the roots of "Daiichi-hienuki" and "Nangun-takedate" from August to September. Not only the weight of the roots but also the growth of the above-ground parts of resistant plants were more vigorous than that of the susceptible varieties. There was no significant difference in the number of lateral roots among varieties, except that the number of lateral roots of "Daiichi-hienuki" were evidently fewer compared to the other varieties. Obser-

vations revealed that the laterals of "Daiichi-hienuki" are thick and they grow horizontally from the upper part of the main tap root. The same phenomenon was also observed in the roots of "Nangun-takedate", though this was not observed in the root systems of "Tokachi-nagaha" and "Kokusô". The laterals of these two latter varieties were thin and quite different from those of the resistant varieties. A comparison of the weight of the roots and the number of laterals is indicated in Table 42.

Table 42. Weight of roots and number of lateral roots of resistant and susceptible varieties.[1)]

Variety	Weight of roots in g per plant		Number of laterals per plant	
	7~8/VIII, '55	6~9/IX, '55	7~8/VIII, '55	6~9/IX, '55
Daiichi-hienuki	27.0	118.3	13.5	12.6
Nangun-takedate	46.6	146.0	22.1	19.5
Kokusô	24.2	19.2	19.0	17.1
Tokachi-nagaha	42.0	49.1	20.3	16.1

1) An average of 4 plants (8 stems).

Number of root nodules The difference in the number of root nodules was remarkable between plants grown in infested and in sterilized soils and also between varieties. The number of root nodules on the damaged plants were fewer than with the healthy plants, and resistant varieties had more root nodules than susceptible varieties. These results are given in Table 43. "Daiichi-hienuki" and "Nangun-takedate" had many root nodules and a large increase was found in the September test. The trend was also evident when represented as the number of root nodules per 1 g of root. The number of root nodules per 1 g of root of the susceptible varieties decreased at the September test and this ratio of decrease was greatest in "Kokusô", which is an extremely early maturing variety. These counts revealed the rise or fall of the activity of the nodule bacteria

Table 43. Number of the root nodules of resistant and susceptible varieties grown in infested and sterilized soils.[1)]

Variety	7~8/VIII, '55		6~9/IX, '55	
	Number of nodules per plant	Number of nodules per 1 g root	Number of nodules per plant	Number of nodules per 1 g root
Daiichi-hienuki	351 (248)[2)]	13.9 (8.6)	1,186 (2,096)	10.7 (16.6)
Nangun-takedate	362 (496)	7.0 (12.0)	880 (1,203)	5.4 (9.3)
Kokusô	94 (161)	3.6 (7.2)	17 (75)	0.8 (4.3)
Tokachi-nagaha	215 (468)	4.9 (12.6)	167 (277)	3.2 (6.0)

1) An average of 2 replications.

2) Data from sterilized soils (control) put in parentheses.

parasitizing the roots of soybean. The number of root nodules on each variety grown in the infested soil and their transition are given in Fig. 11. It was recognized that a plant invaded by larvae caused a decrease in the number of root nodules. Although the cause of this was not clear, a competition phenomenon between the nematode and the root nodule bacteria was postulated.

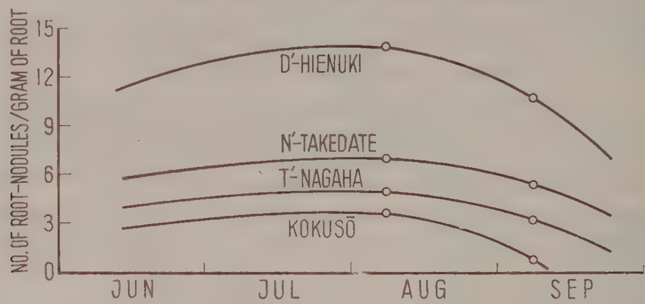

Fig. 11. Transitions of the number of root nodules on the soybean varieties grown in the nematode-infested soil.

This phenomenon of competition between the nematode and the root nodule bacteria as well as the fact that a great many more root nodules were counted on the roots of "Daiichi-hienuki" and "Nangun-takedate", seem to be related to the resistance of the soybean varieties to the nematode.

Number of adult females on the roots Since the root systems of the two resistant varieties are very large as compared with those of the susceptible varieties, and the number of adult females of "Nangun-takedate" in the September test was the largest among all the plants examined, it cannot be easily recognized that the number of adult females per plant of the resistant varieties was fewer than that of the susceptible varieties. However, comparing the number of adult females per 1 g of root, it was found that there were comparatively few females on the resistant varieties as compared with those on the susceptible varieties, and this trend was especially evident for "Daiichi-hienuki". Generally speaking, the number of adult females on each variety greatly increased in the September test comparing with the August count, indicating that the increase in nematode population during this period was very active. The number of the adult females on "Kokusô" decreased in the September test, because this variety matures early and most of the roots begin to decay after maturation. The results of the test are shown in Table 44. The date when the adult females are first recognized on soybean roots in the frame is in the first ten days of July according to past research. The transition of the number of adult females produced on the roots of each variety during the vegetative period was demonstrated and as shown in Fig. 12. The increase ratio in the number of adult females was comparatively small for "Daiichi-hienuki" and "Nangun-takedate" compared with that of the susceptible varieties. The number of adult females of "Kokusô" was considered to

be different from that of the other late-maturing varieties. Since the number of adult females on the roots indicates to some extent the degree of inhibition of plant growth by the nematode, it will be more reasonable to compare the number of adult females per unit weight of root rather than the number of adult females on the entire root system.

Table 44. Number of adult females produced on the roots of resistant and susceptible soybean varieties.[1]

Variety	7~8/VIII, '55		6~9/IX, '55	
	Number of females per plant	Number of females per 1 g root	Number of females per plant	Number of females per 1 g root
Daiichi-hienuki	108	4.3	1,560	14.1
Nangun-takedate	363	7.0	6,607	40.5
Kokusô	638	24.5	366	17.3
Tokachi-nagaha	591	13.4	2,922	56.3

1) An average of 2 replications.

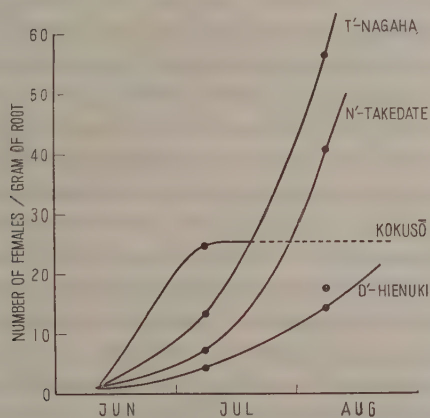

Fig. 12. Transitions of the number of the nematode females matured on the soybean varieties.

7. DISCUSSIONS ON THE RESISTANCE OF SOYBEAN VARIETIES TO THE NEMATODE

The results of the test and observations mentioned above are summarized as follows: (a) "Daiichi-hienuki" and "Nangun-takedate" are definitely varieties resistant to the soybean cyst nematode. (b) This characteristic nature does not disappear even if the varieties are grown under the special environment of a short-day treatment. (c) There is no direct relationship between the number of nematodes invading the plant and the resistance or susceptibility of the variety. (d) There are some individual larvae that die due to an

unknown reason during the development after the invasion. The resistant varieties, comparatively, exhibit many dead larvae. (e) Consequently, the more resistant the variety the fewer adult females mature on the roots. (f) The root growth of resistant varieties is remarkably vigorous and with thick laterals. (g) When the more resistant variety is grown the greater the number of root nodules per unit weight of roots growing in an infested soil. (h) The varieties which have a great number of root nodules have a small number of adult females. The relationship is also valid even within the same variety. Namely, a phenomenon of competition between the root nodule bacteria and the nematode is recognized. (i) "Daiichi-hienuki" is considered to be more resistant than "Nangun-takedate".

From the above, the factors which seem to relate to the resistance of soybean varieties are considered to be the following:

a) Factors inducing the death of the invading larvae during subsequent larval development.
b) Factors activating the root nodule bacteria.
c) Factors stimulating the growth of the root and consequently the growth of the entire plant.

The factors which cause the death of the larvae having invaded the roots are considered to be closely related to the resistance of varieties to the soybean cyst nematode. Many reports (BARRONS, 1939; KÔSAKA, 1947; SHIBUYA, 1952) agree that in the root-knot nematode (*Meloidogyne* spp.) the resistance to the nematode of host plants bears a close relationship with the formation of the giant cell after the invasion of larvae. It has been also recognized that the giant cell is not formed if the invaded larva dies early, or even if the giant cell is formed it soon collapses (KÔSAKA, 1947). In the case of soybean cyst nematode giant cells are formed by the nematode, although they are not so clear as those formed by the root-knot nematode (ICHINOHE, 1955). However, according to a report from Chiba Prefectural Agricultural Experiment Station (1953), the variety "Daiichi-hienuki", which is highly resistant to the soybean cyst nematode, is very susceptible to root-knot nematode (*M. incognita acrita*). In this light, the natural resistance to the soybean cyst nematode seems to be different from that to the root-knot nematode.

Since the activity of the root nodule bacteria is of course limited to the leguminous plants, there are few reports at home or abroad which deal with the relation between nematodes and the root nodule bacteria. A report from the Nagano Prefectural Agricultural Experiment Station (1952, 1954), and a few others, reveal that the number of matured females and the number of root nodules have a reciprocal relationship in the same variety. On the other hand, the symptoms of the diseased plant caused by this nematode, i. e. poor growth and yellowing of the leaves and stems, cannot be seen when soybeans are attacked by the root-knot nematode, even if the damage is severe. Some species of *Heterodera* are parasitic to the sugar-beet, oat, pea or potato and they are reported as not causing the host plant to turn yellow, even though these nematode species are very close to the soybean cyst nematode. The yellowing of the leaves and stems of the soybean plant

affected by the soybean cyst nematode appears quite similar to the symptoms of nitrogen deficiency, therefore, it seems highly probable that the attack of the soybean cyst nematode causes, to a certain extent, a lack of nitrogen. A relation between the soybean cyst nematode and root nodule bacteria is suggested by the fact that the soybean plant needs nitrogen from the root nodule bacteria. Concerning this, it is noted that "Daiichi-hienuki" had the greatest number of nodules and it did not display any symptoms which were seen on the susceptible varieties in these experiments. The author recognized that the yellowish symptom of the soybean plant, even if it is a susceptible variety, was very inconspicuous when a very heavy quantity of nitrogen fertilizer was applied.

The factors which stimulate the growth of the plant could not be neglected. For instance, by giving ample organic manure to susceptible varieties or by cultivating the soil deeply, the growth of the soybean root and consequently the growth of the entire plant is expedited. These methods can even mitigate the damage to some extent in the infested areas. Those varieties suffering severe damage are more prevalent in the early varieties and not usually found in late maturing varieties. This seems to be related to the fact that most of the early varieties are comparatively small whereas the late varieties are large in shape. The date of maturity of "Tan-ryoku", which has recently been proved to be resistant, like "Daiichi-hienuki", to the soybean cyst nematode at Yamagata Prefectural Agricultural Experiment Station (1955), is also late maturing variety. Both of these varieties have been considered to be of little commercial value since the maturing time is too late.

The author suggests that the nature of resistance between soybean varieties could be clarified by comparing the differences in the characteristics of the root tissue or in the parasitic ecology of the nematode among soybean, azuki bean and other beans, as azuki bean and other beans do not suffer severe damage. Or, by clarifying the difference in the parasitic ecology between the soybean cyst nematode and the root-knot nematodes on the same variety of soybean, the solution of the problem might be approached. In fact, the resistance of "Daiichi-hienuki" seems to be different to these two nematodes.

The terms "resistance", "immunity" and "tolerance" seem to have been vague in their meanings. CHRISTIE (1948) used "suitable" and "unsuitable" (host) for susceptible and resistant plants, respectively. DROPKIN (1955, p. 301) divided the degrees of resistance of plants to nematodes into the following three categories: (1) Susceptible; the plant supports the growth and reproduction of the nematode and itself grows poorly or not at all in the presence of the nematode population. (2) Resistant; the plant does not fully support the growth and reproduction of the nematode and at the same time grows well in the presence of the nematode. (3) Tolerant; the plant supports the growth and reproduction of the nematode and grows well itself in the presence of the nematode. JONES (1954, pp. 350~351) classified the resistance of plants to *Heterodera rostochiensis* as follows:

1. Absolutely resistant. Not invaded.
2. Partially resistant. Invaded.
    a. Larvae fail to develop.

b. Larvae develop but fail to mature.

c. Larvae mature but the females are reduced in numbers and not very prolific.

3. Susceptible. Large numbers of highly prolific females produced.

"Daiichi-hienuki" and "Nangun-takedate" are the varieties to be classified under "Tolerant" of DROPKIN, or "2 (c)" of JONES. Therefore, these varieties are to be called "tolerant", not "resistant", to the soybean cyst nematode in the strict sense of the term.

VII. Studies on the Control Measures of the Soybean Cyst Nematode

1. HISTORICAL REVIEW

The first comprehensive account on the control measures of the soybean cyst nematode was made by ITÔ (1921). He indicated the undernoted should be recommended to control the soybean disease caused by this nematode.

A. Indirect control measures.

a. Rotation. A longer than two-year rotation should be introduced to the prevailing areas. Three-year rotations can be better than a two-year rotation and seem satisfactory.

b. Manuring. Since the damage to plants by this nematode seems severe in infertile soils, ample fertilizers should be applied to the soil.

c. Plowing in the fall. It will be effective to control the nematode to plow in the fall so that the infested soil is exposed to low temperature.

d. Resistant varieties. Varieties which have the resistance to the nematode such as "Ôyachi" should be used.

e. Agricultural tools should be cleaned after every use to prevent the uninfested fields from being contaminated with the nematode.

f. Good quality seed should be used.

B. Direct control measures.

a. The diseased plants should be collected and burnt.

b. The sacrifice plant (or trap crop) is said to be effective for control of this nematode.

c. Experiments on the chemical control of this nematode are now under way.

It has long been emphasized that rotations are the most reliable means to control the soybean cyst nematode, and many works in this connection have been made mainly in the Hokkaido Agricultural Experiment Station since about 1920. IWATA (1941) made these experiments and he reported the results. According to him, 5- and 6-year rotation systems, such as suguar-beet–wheat–corn–potato–soybean croppings or flax–oat–pea–corn–soybean for a 5-year rotation system, and potato–sugar-beet–wheat–flax–buckwheat–soybean for a 6-year rotation system, were found almost perfect both for obtaining good yields of soybeans and for starving the nematodes. The results indicated that no adult females were counted on the soybean roots. The same data ravealed that in most cases a 3-year rotation such

as wheat–potato–soybean or pea–flax–soybean croppings and 4-year rotation system such as oat–pea–potato–soybean or corn–red clover–sugar beet–azuki bean croppings were found unsatisfactory for controlling this nematode, although comparatively high yields of soybean were obtained as compared with successive soybean- or azuki bean-crops. IWATA also stated that since Spanish runner beans are resistant to this nematode, a combined planting of this plant and soybean or azuki bean in the same field shows a better result than a single cropping of soybean.

As to the varieties resistant to the soybean cyst nematode, according to KATSUFUJI (1919) in Hokkaido, varieties "Yoshioka" and "Ôyachi" were said to be resistant to this nematode while varieties "Kotsubu" and "Mejiro" were susceptible.

Experiments on the chemical control of this nematode have been made by many workers, mostly in the Hokkaido Agricultural Experiment Station. The chemicals first applied to the experiments in or about 1935 were carbon disulphide, calcium cyanamide, and chloropicrin. Since about 1950 several new nematicides such as "D-D", ethylene dibromide, "Vapam", "VC–13", methyl bromide, "Mylone", and "DBCP" have been used to ascertain their effect in controlling this nematode. Two of them, "D-D" and ethylene dibromide are now in general use in this country.

2. STUDIES ON THE CONTROL OF THE NEMATODE BY THE CROPPING METHOD

Rotations including resistant plants In order to control the soybean cyst nematode, it should be particularly emphasized that a rotation is still one of the most reliable measures which is effective for reducing the damage. According to the studies which were made by the Hokkaido National Agricultural Experiment Station, a 5- or 6-year rotation system (cropping soybean in every fifth or sixth year) is found to be almost perfect to control this nematode, if we are careful to prevent introducing contaminated soil from other fields. In most cases, 3- and 4-year rotations are found unsatisfactory if effective control is required, on the other hand, there are several cases in which even after more than five years of growing non-host plants the yellowish dwarf patches appeared during the first year when soybeans were reintroduced. In such cases, the dispersal of the nematode cysts might have occurred from agricultural implements, particularly when the plough is used carelessly. The author thinks that in most cases the disease is spread in this way.

The most effective means to control this nematode is to use rotations involving as many trap crops as possible. According to the author's studies which have been mentioned in Chapter V, a few leguminous plants such as red clover, alfalfa, and peas seem to be useful for this purpose. The experiments showed a remarkable reduction in the nematode populations by planting such crops rather than fallowing or planting host plants. The effect of growing kidney beans on the nematode population is peculiar as the experiments revealed that the plots where kidney beans grow have less population at the end of the season than the initial population levels, though a large increase in the nematode popula-

tions occur in the adjacent plots where soybeans and azuki beans grow. These results mean that kidney beans are effective to reduce nematode population in the soil, and they can be used as a trap crop in spite of the fact that this plant was supposed to be one of the host plants of the soybean cyst nematode.

The reduction ratio of the nematode population from the initial level, by one cropping of each of several crops, was derived from the results of experiments made during the four years, 1954~1957, and as already mentioned on pages 35~39. These results are given in Table 45. It seems difficult to find a trend from the results of these experiments, because decreases or increases in the nematode populations are greatly dependent on the conditions under which the experiments are undertaken. These conditions are: an initial nematode population level; physical factors such as soil temperature, soil moisture and nature of soil; scale and length of experiment; technique to determine the nematode populations; quantity and nature of inoculum; growth period of plant, and the plant species. The necessity for uniform and controlled techniques can be judged also from the considerable range of reduction ratios as given in Table 45.

Table 45. Reduction ratio of the nematode population from the initial level, following one cropping of plants, derived from the results of experiments made during 1954~1957.

Plant grown	Number of tests	Range of reduction ratios (%)	Average of reduction ratio (%)
Pea	3	53~92	77
Kidney bean	8	64~94	74
Red clover	6	52~82	68
(Fallow)	7	4~80	49
Potato	3	27~64	44
Soybean	6	−61~86	10

The control of this nematode by reducing the population in the soil through rotations has been actually practiced by the farmer to some extent without knowing it. In fact, kidney bean, red clover, and peas are very commonly cultivated in Hokkaido.

Cropping resistant soybean varieties Varieties of soybeans highly resistant to the soybean cyst nematode seem to be very few. Out of hundreds of soybean varieties tested, four varieties were found comparatively resistant to the nematode in a certain district of the northern part of Honshû (mainland), where this nematode is also prevalent. The varieties are "Daiichi-hienuki", "Nangun-takedate", "Geden-shirazu", and "Tan-ryoku".

These resistant varieties indicate an almost healthy appearance and they have a good yield in spite of harboring fairly large numbers of females on their roots. They are so resistant to this nematode that they yield approximately 80 per cent of uninfected plants, while the yield of susceptible varieties planted in the same field are reduced by 80 to 100

per cent.

However, there are some difficulties in the use of the resistant varieties. One is that they all mature extremely late, even at their original habitat, and in most cases in Hokkaido they fail to mature. According to the record of the Kariwano Branch of Tôhoku National Agricultural Experiment Station in Akita Prefecture, the flowering date of "Daiichi-hienuki" is August 9th, the date of maturing is October 25th with a growth period of 152 days. The maturation is extremely late among the varieties of the same prefecture. As to "Nangun-takedate", the Nanbu Branch of Aomori Prefectural Agricultural Experiment Station reported that the flowering date is August 10th, maturity is October 25th and which is the latest among the superior varieties in that prefecture. In the Yamagata Prefectural Agricultural Experiment Station, the date of maturity is recorded as November 7th for "Daiichi-hienuki" and "Tan-ryoku", in fact being too late to mature in this prefecture.

It is also commonly said that the quality of seed of some resistant varieties is inferior. For example the seed of "Daiichi-hienuki" is distorted, elliptical in shape, green in colour and the commercial value of this seed is considered low. Of these four varieties mentioned, "Geden-shirazu" seems most useful in its high resistance to the soybean cyst nematode and in the production of good quality of seed. Attempts have been made to purify the lineage as well as to shorten the growth period by Masazi ISHIKAWA in the Kariwano Branch of Tôhoku Nat'l Agr. Expt. Station. Very recently this variety has come into general use in Tôhoku District under the new name of variety "Tôhoku No. 6".

In 1951 the author studied the degree of female infestation on the roots grown in an infested field using 14 representative soybean varieties in Hokkaido. The relative index system which was proposed by SMITH and TAYLOR (1947) for rating the root-knot nematode galls, was employed to determine the degree of female infestation. The results indicated that the degree of the female infestations varied to a great extent according to the varieties tested, though correlations between the female infestation indices and the varietal characteristics such as maturity, colour of seed and the size of seed are not clear as is seen in Table 46. (MUKASA and ICHINOHE, 1952)

As mentioned previously, hitherto, no "completely resistant" varieties on which no adult females are present, have been found. In 1956, the author made a test on varietal resistance by using the soybean variety "Laredo" and five other varieties at Kotoni, Hokkaido. "Laredo" has been reported to be extremely resistant to the root-knot nematode (*Meloidogyne incognita acrita*) by SMITH and TAYLOR (1947). The seeds of this variety were obtained by courtesy of Mr. Masazi ISHIKAWA and planted in the nematode-infested field. The test revealed that "Laredo" bore very few females on its root compared to the other varieties tested. It was also noted that this variety was extremely late in maturing and failed to mature within the growing season in Hokkaido. The number of adult females and the root nodules counted on the roots of "Laredo" are given in Table 47 and compared with those of the other five varieties tested.

Table 46. The indices of the female infestation on the roots of the 14 representative soybean varieties in Hokkaido.1)

Variety	Female infestation index (%)				
	I	II	III	IV	Mean
Yoshioka-dairyû	72	66	73	75	72.0
Nakate-hikarikuro*2)	63	80	68	68	69.8
Nukanai-daizu*	68	70	66	67	67.8
Wase-tsurunoko*	64	61	69	62	64.0
Akazaya No. 1*	62	55	63	61	60.3
Shiro-kotsubu*	62	51	52	70	58.8
Gokuwase-chishima	38	67	60	54	54.8
Tokachi-nagaha	59	49	55	49	53.0
Tokachi-hadaka	51	51	53	50	51.3
Ôyachi No. 2	43	51	62	46	50.5
Rankoshi	48	44	49	51	48.0
Wase-kurosengoku*	47	49	46	47	47.3
Ishikari-shiro No. 1	41	39	57	49	46.0
Wase-hadaka	30	32	46	51	39.8
L. S. D. (5% level)					9.1

1) Examinations made August 18~19th, 1951, on 25 to 50 plants of each variety in each replication.

2) An asterisk means the comparatively late-maturing variety.

Table 47. Number of adult females and the root nodules counted on the roots of "Laredo" and the five soybean varieties tested in 1956.1)

Variety	Number of nodules per plant			Number of females per plant		
	2/VIII	4~5/IX	28~29/IX	2/VIII	4~5/IX	28~29/IX
Laredo	34	47	137	5	5	1
Daiichi-hienuki	73	240	639	428	676	448
Nangun-takedate	37	90	291	722	804	694
Tokachi-nagaha	29	77	188	553	143	185
Date-shindaizu	26	112	180	258	312	272
Black hawk	12	57	68	310	280	88

1) An average of 6 plants.

As is indicated in Table 47, "Laredo" indicated almost no adult females and comparatively few root nodules on its root. Also the root system of this variety contained much fewer, but thicker, lateral roots than those of the other varieties tested, though these lateral roots did not grow vertically, rather horizontally, resulting in quite a different

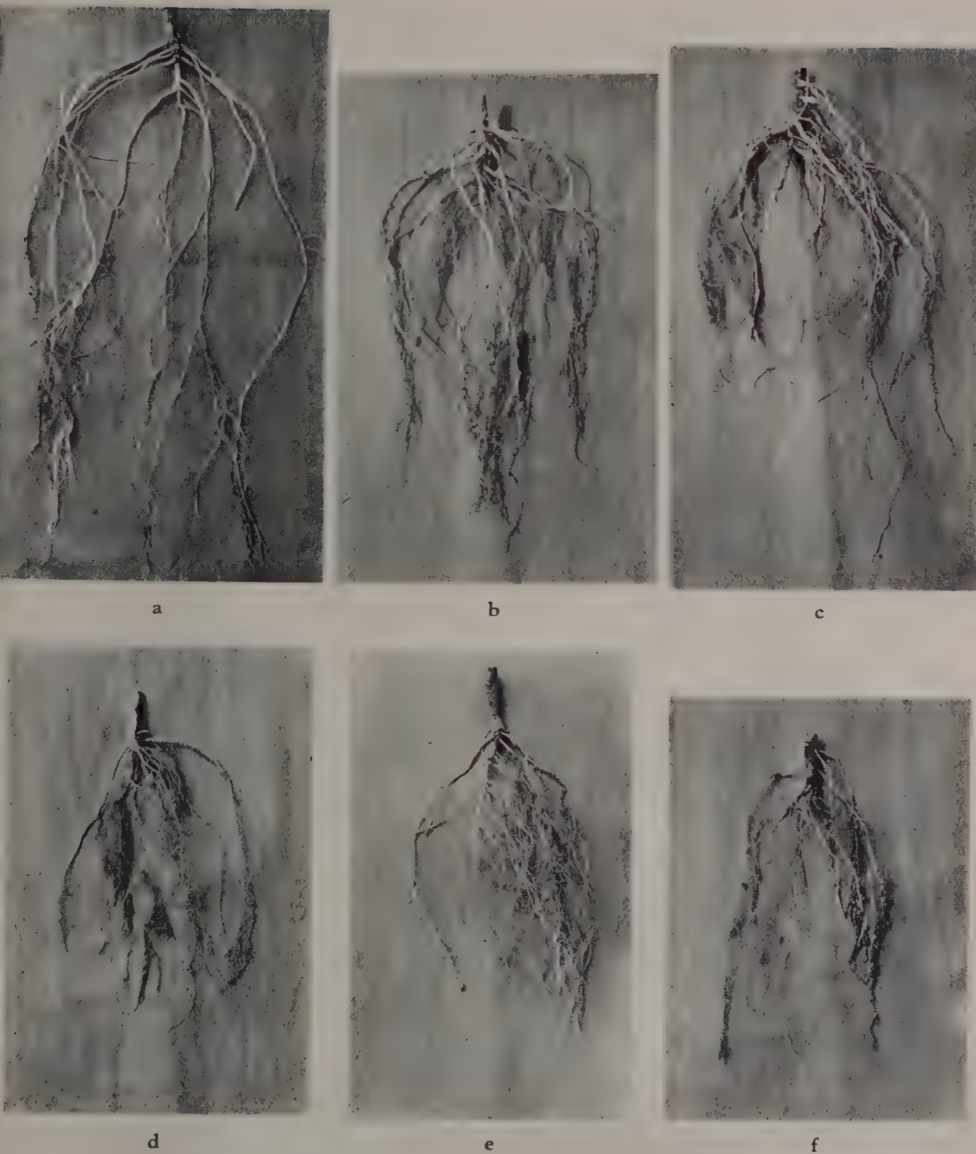

Fig. 13. Photographs of roots of six soybean varieties tested in 1956. Pictures were carefully prepared to be reduced to the same scale. (a) Laredo (b) Daiichi-hienuki (c) Nangun-takedate (d) Tokachi-nagaha (e) Date-shindaizu (f) Black hawk.

appearance of the root system from those of the other varieties. Photographs of the root systems of the varieties tested are given in Fig. 13. The author thinks that "Laredo" cannot be used generally because the seed is quite inferior in its characteristics, however, the nature of the resistance of this variety is worthy of investigation in the future.

3. EXPERIMENTS ON THE CHEMICAL CONTROL OF THE SOYBEAN CYST NEMATODE

The soybean cyst nematode is comparatively easy to control with moderate applications of standard nematicides such as "D–D" or ethylene dibromide, but the complete eradication of this nematode seems quite difficult. "D–D" contains 1,3-dichloropropene as the active ingredient. It is also apparent that the increase of the nematode population in soil is so rapid that even if the chemicals reduced the nematode population to a low level, a high population will again be built up by one or two croppings of soybean. The experiments which have been made at the Hokkaido National Agricultural Experiment Station since 1950 have proved that 30 l/10 ares "D-D" (300 pounds per acre) gives very effective control. 30 l/10 ares ethylene dibromide ("Nemafume" W-20) was also proved effective, though this chemical seemed slightly less effective compared at the same dosage of "D-D". The undernoted are the results of the experiments conducted since 1950 in Hokkaido by the author.

Obihiro field experiment in 1950 Chemical control experiments with "D-D" and chloropicrin were made in the nematode-infested field at Obihiro, Hokkaido in 1950.

These chemicals were used at the rate of 20 l per 10 ares (200 pounds per acre) and were proved effective for increasing the height of plant, the number of pods, and the yield of soybean. The nematode population in the soil at harvest-time was significantly decreased in the treated plots, particularly in the plots treated with chloropicrin. The results are given in Table 48.

Table 48. Soybean cyst nematode control experiment with "D-D" and chloropicrin (1950 Obihiro field experiment).[1)]

Treatment	Height of plant	Number of pods per plant	Yield of soybean converted per 10 ares (kg)	Nematode population at harvest-time[2)]
"D-D" 200 lbs./acre	70.6**[3)]	68.7**	54.7**	249**
Chloropicrin 200 lbs./acre	68.5**	64.6**	54.7**	137**
Check	62.8	55.5	48.7	297

1) An average of 3 replications, 20 plants for each replication.

2) Nematode population expressed by the number of viable cysts in soil one-millionth acre and 15 cm deep in volume.

3) Two asterisks or one asterisk mean that the difference between "check" and the treatment indicated is significant at 1% level or at 5% level respectively.

Kotoni pot experiment in 1951 Chemical control experiments were conducted at Kotoni, Hokkaido in 1951 with "D-D", chloropicrin, methyl bromide, calcium cyanamide, and "Urea" (commercial nitrogen fertilizer). These chemicals were applied to pots which were inoculated with the nematode cysts at the same population level.

All the chemicals except chloropicrin were effective in control, particularly "Urea" which gave an exceptionally good effect produced later high yields of soybean. The nematode populations in the soil at harvest-time were moderately, sometimes highly, decreased by the use of the chemicals, except for "Urea" in which the final nematode population level was considerably higher than that of the non-treated pot. It was indicated that "Urea" is very effective to increase the soybean yield, but at the same time results in building up a high nematode population level. The results are given in Table 49.

Table 49. Soybean cyst nematode control experiments with "D-D", chloropicrin, methyl bromide, calcium cyanamide, and "Urea" (1951 Kotoni pot experiment).[1,2,3,4]

Treatment	Height of plant (cm)	Number of pods per plant	Weight of seed per plant (g)	Nematode population at harvest-time[5]
"D-D" 200 lbs./acre	53.4	53.3	15.8**	2.3*
"D-D" 400 lbs./acre	57.3**	60.3*	15.6**	1.9**
Chloropicrin 200 lbs./acre	58.4**	17.3	3.0	2.7*
Chloropicrin 400 lbs./acre	58.1**	33.0	4.2	6.8
Methyl bromide 100 lbs./acre	58.3**	47.0	11.2**	4.0*
Methyl bromide 200 lbs./acre	55.1*	35.3	7.3**	1.3**
Calcium cyanamide 180 kg/acre	54.8*	45.3	7.9*	5.3
Calcium cyanamide 750 kg/acre	58.2**	78.7**	10.8**	7.6
"Urea" 750 kg/acre	56.8	125.7**	25.6**	21.9**
Check	49.2	22.7	3.8	11.9
Non-inoculated	56.0**	82.0**	17.6**	—

1) An average of 3 replications.
2) The soil was not sealed after all application of the chemicals.
3) Methyl bromide was applied mistakenly without a seal.
4) Asterisks are the same as noted in Table 48.
5) Nematode population expressed by the number of viable cysts per 10 g dry soil.

Memuro field experiment in 1952 Chemical control experiments with "D-D", chloropicrin, ethylene dibromide (this chemical was technically pure as no commercial "EDB" was available at that time), "Urea" (60 per cent ingredient) and calcium cyanamide were set up in 1952 in the infested field at Memuro, Hokkaido.

The treatments and results are given in Table 50. "D-D" and ethylene dibromide were remarkably effective to control the soybean cyst nematode. The nematode population in the soil at harvest-time was found to have increased in all of the treated plots compared with the non-treated plots (check).

Additional experiments were made in the adjacent plots to test the effect of the organic phosphorus insecticide "Folidol" for control of this nematode. The result indicated that the application of a moderate dosage of this chemical to the soil prior to planting was of no use in controlling the nematode.

Table 50. Soybean cyst nematode control experiment with "D-D", chloropicrin, ethylene dibromide, "Urea", and calcium cyanamide (1952 Memuro field experiment).[1]

Treatment	Height of plant (cm)	Number of pods per plant	Yield of seed per plot (g)	Yield of seed convert. per 10 ares (kg)	Nematode population at harvest-time[2]
"D-D" 300 lbs./acre	46.5**	60.3**	1261**	315.3	4.7
Chloropicrin 200 lbs./acre	46.1**	34.7**	365**	91.3	8.3**
Ethylene dibromide 200 lbs./acre	55.3**	62.0**	1504**	376.0	3.9
"Urea" 180 kg/acre	40.5**	23.0**	435**	108.8	8.8**
Calcium cyanamide 180 kg/acre	36.8**	21.0**	305**	76.3	6.5**
Check	28.9	13.0**	167	41.8	3.9

1) An average of 3 replications.

2) Based upon the number of viable cysts per 10 g dry soil.

Shintoku field experiments in 1953 & 1954 "D-D" and chloropicrin were applied to an infested field at Shintoku, Hokkaido in 1953.

The application of "D-D" at the rate of 40 l per 10 ares (400 pounds per acre) gave a better result than 20 l per 10 ares. The application of 60 l per 10 ares of chloropicrin (600 pounds per acre) was better than 30 l per 10 ares. It was shown that the effects of "D-D" (200 pounds and 400 pounds per acre) are almost the same as the effects respectively of chloropicrin at 300 pounds and 600 pounds per acre. The female-infestation indices on the roots examined at harvest-time were significantly decreased in the "D-D" treated plots. The results are given in Table 51.

In the next year, half of the experiment plots employed in 1953, i. e. two replications,

Table 51. Soybean cyst nematode control experiment with "D-D" and chloropicrin (1953 Shintoku field experiment).[1]

Treatment	Height of plant (cm)	Number of pods per plant	Weight of seed per plant (g)	Female-infestation index (%)
"D-D" 200 lbs./acre	38.7**	23.1**	8.3**	46**
"D-D" 400 lbs./acre	37.7**	26.3**	11.8**	45**
Chloropicrin 300 lbs./acre	38.7**	22.9**	8.7**	80
Chloropicrin 600 lbs./acre	39.9**	28.8**	10.6**	64
Check	28.0	6.9	1.8	78

1) An average of 4 replications, using 15 plants in each replication.

were used in order to find the effects of the chemicals in the second year. The applications of "D-D" at the rate of 20 and 40 *l* per 10 ares (200 and 400 pounds per acre) respectively and chloropicrin 30 and 60 *l* per 10 ares (300 and 600 pounds per acre) respectively in the previous year showed no effect in the control of the soybean cyst nematode. The results are indicated in Table 52.

Table 52. Effects of "D-D" and chloropicrin on the growth and yield of soybean in the second year (1953 & 1954 Shintoku field experiments).[1]

Treatment	Height of plant (cm)		Number of pods per plant		Weight of seed per plant (g)	
	1953	1954	1953	1954	1953	1954
"D-D" 200 lbs./acre	37.4	37.0	22.9	9.0	8.8	1.4
"D-D" 400 lbs./acre	40.6	45.0	29.1	15.0	13.3	1.7
Chloropicrin 300 lbs./acre	39.3	29.5	23.1	10.5	8.0	1.3
Chloropicrin 600 lbs./acre	40.3	37.5	27.5	7.5	11.9	0.9
Check	27.5	38.0	6.8	10.0	1.8	0.8

1) An average of 2 replications, using 15 plants in each replication.

Shimamatsu field experiment in 1957 "D-D", "Vapam" (31% active), "EDB W-20", "Telone" and "Fumazone" were used and their effects in the control of soybean cyst nematode were examined in 1957 at Shimamatsu, Hokkaido.

The application of "Vapam (1)" at the rate of 45.5 *ml* per square meter indicated no effect, while the same dosage of "Vapam (2)", "Telone" and "Fumazone" at the rate of 30 *l* per 10 ares (300 pounds per acre) were found effective for the control of this

nematode. The application of "EDB W–20" at the rate of 30 l per 10 ares (300 pounds per acre) seemed less effective than that of the same dosage of "D-D". The results are shown in Table 53.

Table 53. Effects of "Vapam", "EDB W–20", "Telone", "Fumazone" and "D-D" for the control of soybean cyst nematode (1957 Shimamatsu field experiment).[1]

Treatment		Height of plant (cm)	Number of pods per plant	Weight of seed per plant (g)
"Vapam (1)"	45.5 ml/sq. meter	40.2	20.6	5.2
"Vapam (2)"	45.5 ml/sq. meter	43.2	29.3*	8.9*
"EDB W–20"	300 lbs./acre	45.2	24.7	7.3
"Telone"	300 lbs./acre	45.8	30.1*	9.8*
"Fumazone"	300 lbs./acre	46.6	29.2*	10.0*
"D-D"	300 lbs./acre	43.5	29.9*	9.4*
Check		44.7	21.8	5.8

1) An average of 4 replications, using 10 to 20 plants in each replication.

4. THE PRESENT SITUATION AND THE FUTURE PROSPECTS OF THE CHEMICAL CONTROL OF THE SOYBEAN CYST NEMATODE

As has been shown by several experiments, the most effective nematicides which can be applied to the field to control the soybean cyst nematode are fumigants which contain 1,3-dichloropropene, or ethylene dibromide, as the active ingredients.

However, there are some applicational difficulties in the use of these chemicals for the control and also they should not be expensive. The above chemicals cost more than 6,000 yen per 10 ares (approximately US $ 67 per acre) until 1958, and about 4,500 yen (approximately US $ 50 per acre) in 1960. Since soybeans are one of the crops which have the lowest cash values in this country, the application of these chemicals to the field is still uneconomical and impracticable. According to the agricultural statistics of Hokkaido in 1953, the average yield of soybean per 10 ares was 2.46 bales (1 bale contains about 1.9 bushels) and the market price of 1 bale was 3,520 yen. This means that the farmers in Hokkaido are making 7,600 yen average gross profit by a soybean cropping of 10 ares (one-fourth acre).

There are several reasons why the farmers had to culture soybeans in Hokkaido so extensively in spite of low cash value, one of the reasons being that the soybean plants are easy to raise even in comparatively sterile soil in so far as it is free from nematodes. The second reason is that the market price of soybean seed is rather stable compared to other kinds of beans. Thus the farmers are used to growing soybeans successively on the same land, and, as a result, the nematode population level in the soil

was increased year after year, and the crop damages becoming more serious.

The other difficulty in the use of the chemicals is that there has been no good injection equipment until 1958 which could be used on large areas of soybean. The farmers cultivate soybeans on a large scale (i. e. one property of soybeans extends several tens of ares or sometimes even several hundreds of ares) in Hokkaido and the application of the chemicals to the soil must be reasonably simple and easy. The hand soil injector is of no use from this point of view in Hokkaido. It was necessary to apply fumigants by means of a specially equipped tractor which could deliver the liquid to the soil through tubes attached to narrow cultivator tines. This type of fumigator, a "trailer type soil fumigator" was developed in 1959 and used for the first time in Hokkaido.

Fig. 14. View of soybean field in Hokkaido. Photographed in September 1952, at Memuro, Hokkaido.

Fig. 15. The newly-made "Gandy"-type soil fumigant distributor which is delivering "DBCP" granules into the soil. Photographed on August 10th, 1960, at Egasaki, Chiba Prefecture.

The third difficulty is in climatic conditions. In Hokkaido, soybean is usually seeded in the middle ten days of May, a date determined mainly by the temperature of the soil. Generally the average soil temperature reaches 10°C at seeding-time, on the other hand, the fumigants should be applied to the soil two or three weeks prior to the date of seeding. Therefore, the soil temperature at the time of application is inevitably below 10°C and, owing to such low temperatures, it is found that the fumigants are sometimes ineffective to kill the nematode. Naturally it is hoped to have chemicals which have no phytotoxicity and can be applied whenever the farmers desire.

New materials containing 1,2-dibromo-chloro-propane, abbreviated as "DBCP", are being extensively tested in this country against the soybean cyst nematode and other nematode species, and the results so far obtained seem exceptionally good. These materials are tolerated by many crop plants, except for species such as tobacco and onion, and it can be applied even after planting in the case of soybeans. This new chemical was first reported by McBETH (1954) and RASKI (1954) in U. S. A. and it is said that this material will undoubtedly be one of the best nematicides. Another great benifit of this chemical is that granular formulations are available, and which can enable the farmers to apply "DBCP" to the soil using most kinds of fertilizer distributors. The granules can even be mixed with the fertilizer and applied at the same time. The granular "DBCP" is very commonly used in U. S. A. and the procedure of application is quite easy by the use of simple tractor distributor equipment. One of these types of soil fumigant distributor is called the "Gandy" and this type of machine has been newly developed in this country with extensive testing in progress.

In this country, the use of nematicides has been fairly well established in certain areas and proved successful for certain kinds of crops. However, there is, of course, a limit to the profitable application of the chemicals from the view-points of price of the chemical, machines, timing of application, and cash value of the harvested product. Less expensive and non-phytotoxic nematicides for the plants of low cash values like soybeans are desirable.

VIII. Summary

This paper describes the morphology, bionomics and control measures of the soybean cyst nematode, *Heterodera glycines*, made through studies by the author since 1949, mainly at the Hokkaido National Agricultural Experiment Station.

1. The morphology of each life stage of this nematode is described. The first molt takes place by the first-stage larva inside the egg-shell. The second-stage larva invades the root, molts, and develops into the third-stage larva. The sexes of the larvae can be differentiated in the third-stage larva by the development of the genital primordium. A single specimen of the third-stage male larva is observed possessing internally a cellular string located laterally beneath the cuticle, as reported by RASKI (1950). The third-stage larva of both sexes molts and develops into the fourth-stage larva. The final molt takes place with the fourth-stage larva, which develops into the adult. The number of eggs

deposited in the egg sac varies from a few to about 200. The number of eggs contained within a single cyst ranges from 95 to 478, with an average of 262 eggs.

2. The systematic description of *Heterodera glycines* is given. *H. glycines* belongs to the so-called "*schachtii* group", viz., a lemon-shaped cyst with a zig-zag cyst-wall pattern and brown knobs, closely resembling *H. trifolii*, from which *H. glycines* differs in the presence of males, in the distance between the base of the stylet and the dorsal oesophageal gland orifice in the second-stage larva, and in the selection of host plants.

3. No difference in the number of root-infesting females is found between infested soybean and azuki bean, while kidney bean is found to be unsuitable as host plant of this nematode, since this plant shows a very much smaller number of adult females on the root than exhibited by soybean and azuki bean plants. The rate of larval development inside the root tissue is almost identical in the 3 susceptible plants, though the young females in the root tissue of kidney bean develop very poorly being small in size and producing few eggs. The female hardly protrudes from the surface of the root. It was ascertained that the larvae of this nematode invade some plants which had hitherto been regarded as non-host plants, and they developed into partly-grown larvae, which however, could not reach the adult stage in the root tissue. These host plants are Spanish runner bean, lima bean, alsike clover, etc.

4. The possible number of generations per year of the soybean cyst nematode is computed from the experiments made in 1952 and 1953. The number of days required for the completion of one generation of this nematode varied with the soil temperature and ranges from 24 to 41 days. The average soil temperature at a depth of 5 cm during each generation ranged from 17.8°C to 23.3°C. The relationship between the rate of the development of the nematode and the average soil temperature is studied, and it was shown that the threshold temperature, the minimum temperature at which the nematode ceases to develop, is 10°C. The accumulated effective temperatures necessary to complete one generation varies from 304 to 320 with an average of 313 day-degree. The possible number of generations is obtained by dividing the total effective temperature during the vegetative period of soybeans in Hokkaido by the accumulated effective temperature of 313 day-degree; it is found to be 3.8 at a depth of 5 cm and 3.4 at a depth of 30 cm. It is concluded that three generations are the maximum per year which can be completed by the nematode on soybean roots in Hokkaido, so far as other conditions are favourable for it to develop.

5. Grafting is made with host and non-host plants of this nematode. Out of 84 trial grafts of various combinations, only 9 lima bean plants grafted onto kidney bean stocks were successfully obtained. The nematodes which invaded the root of the grafted plants and developed for several weeks, are evidently small in size and they produce few eggs, resembling those maturing on normal kidney bean plants.

6. To investigate the relationships between plant damage and nematode population in the soil at the time of harvest, several experiments were conducted at different locations in Hokkaido. It is evidently shown that a high negative correlation exists between the

yield of soybeans and the nematode population in the soil.

7. The nematode population is studied on growing host and non-host plants. It is generally shown that a crop of soybean maintains the initial nematode population level, while fallowing and non-host plant crops result in a moderate decrease in the final nematode population. It is important to have discovered that the final nematode population levels are highly decreased by growing kidney bean which was supposed to be a host plant of this nematode.

8. Experiments were made during 1955 to 1957 in the successively soybean planted field plot where the soybean cyst nematode was first found in 1955. The relationship between the nematode population and the soybean growth in the plot is studied and it is found that they are highly correlated. Particularly, the height and weight of the plant and the weight of seed per plant are most closely correlated with the nematode population at the time of harvest. An equation of regression between the weight of seed per plant and the nematode population was computed as follows:

$$Y = 14.1 - 0.233\,X$$

The annual transitions of the nematode population and the plant symptoms in this plot are very remarkable and showing a striking increase and extension.

9. Four soybean varieties are studied and compared concerning the growth and yield in the nematode-infested soil. The morphological and ecological characters of their roots and the development of the nematode inside the root tissue are also studied. The soybean plant varieties "Daiichi-hienuki" and "Nangun-takedate" do not suffer much damage from this nematode, and this characteristic does not disappear even if they are grown under special environmental conditions of a short-day treatment. There is no direct relation between the number of nematodes invading the roots and the resistance or susceptibility of the soybean varieties, but some of the larvae within the root are found to have died during the development. The number of dead larvae seems to be related to the varietal resistance to the nematode. The root-growth of the resistant varieties is remarkably vigorous with thick lateral roots. The more resistant variety has the greater number of root nodules per unit weight of roots when growing in infested soil. The varieties with the greater number of root nodules have the fewer number of adult females. From these results, a phenomenon of competition between the root nodule bacteria and the nematode is suspected.

10. Rotations including resistant plants are recommended to control the soybean cyst nematode. Experiments showed remarkable reductions in the nematode population by planting some of the leguminous crops such as red clover, alfalfa, and peas as compared with fallowing or planting of host plants. It was further proved that kidney beans are effective for reducing the nematode population and will serve as a trap crop.

11. There are several soybean varieties "tolerant" to the nematode, however, they mature extremely late and thus cannot be used in Hokkaido with the short period of growth conditions present. The author found that "Laredo" soybean variety indicated only a few adult females on its root as compared with other varieties. Though "Laredo"

riety is extremely late-maturing and the seed characteristics are quite inferior, the nature resistance of this variety seems worthy of further investigation.

12. Several experiments with chemicals for the control of this nematode prove that oderate applications of standard nematicides such as "D-D" or ethylene dibromide are ective. Applications of "D-D" at the rate of 20 to 30 l per 10 ares (200 to 400 pounds r acre) are very effective, but no effects were shown in the successive cropping of ybean in 1953 and 1954 experiments.

13. There are some problems in the use of chemicals in the practical control of this matode. The price of chemicals and machines to apply the chemicals to the soil, matic conditions at the time of application and the low cash value of soybean should carefully studied. It is necessary to produce and develop less expensive and non-ytotoxic nematicides for use in this country.

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X. Literature Cited

BARRONS, K. 1939. Studies of the nature of root knot resistance. Jour. Agr. Res., 58, pp. 263～271.

Chiba Pref. Agr. Expt. Sta. 1953. Tests on the characteristic nature of soybeans in 1953. (not for publication)

CHRISTIE, J. R. 1949. Host-parasite relationships of the root-knot nematodes, *Meloidogyne* spp. III. The nature of resistance in plants to root knot. Proc. Helminth. Soc. Wash., 16 (2), pp. 104～108.

CHRISTIE, J. R. 1959. Plant nematodes, their bionomics and control. Agr. Expt. Sta., Univ. Florida, Gainesville, Fla. 256 pp.

COOPER, B. A. 1955. A preliminary key to British species of *Heterodera* for use in soil examination. Soil Zoology, edited by D. K. McE. Kevan, London, pp. 269～280.

CRITTENDEN, H. W. 1954. Factors associated with root-knot nematode resistance in soybeans (Abstract). Phytopath., 44 (7), p. 388.

DONCASTER, C. C. 1953. A study of host-parasite relationships. The potato-root eelworm (*Heterodera rostochiensis*) in black nightshade (*Solanum nigrum*) and tomato. Jour. Helminth., 27 (1/2), pp. 1～8.

DROPKIN, V. H. 1954. Infectivity and gall size in tomato and cucumber seedlings infected with *Meloidogyne incognita* var. *acrita* (root-knot nematode). Phytopath., 44 (1), pp. 43～49.

DROPKIN, V. H. 1955. The relations between nematodes and plants. Exp'l Parasit., 4 (3), pp. 282～322.

EPPS, J. M. and A. Y. CHAMBERS 1958. New host records for *Heterodera glycines*; including one host in the Labiatae. Pl. Dis. Repr., 42 (2), p. 194.

EPPS, J. M. and A. Y. CHAMBERS 1959. Mung bean (*Phaseolus aureus*), a host of the soybean cyst nematode (*Heterodera glycines*). Pl. Dis. Repr., 43 (9), pp. 981～982.

FENWICK, D. W. 1940. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. Jour. Helminth., 18 (4), pp. 155～172.

FENWICK, D. W. and M. T. FRANKLIN 1951. Further studies on the identification of *Heterodera* species by larval length. Estimation of the length parameters for eight species and varieties. Jour. Helminth., 25 (1/2), pp. 57～76.

FILIPJEV, I. N. and J. H. SCHUURMANS STEKHOVEN 1941. A manual of agricultural helminthology. E. J. Brill, Leiden, Holland. 878 pp.

FRANKLIN, M. T. 1940. On the specific status of the so-called biological strains of *Heterodera schachtii* SCHMIDT. Jour. Helminth., 18 (4), pp. 193～208.

FRANKLIN, M. T. 1951. The cyst-forming species of *Heterodera*. Commonwealth Agr. Bur., Farnham Royal, Bucks, England. 147 pp.

FUJITA, K. and O. MIURA 1934. On the parasitism of *Heterodera schachtii* SCHMIDT on beans. Trans. Sapporo Nat. Hist. Soc., 13 (3), pp. 359～364.

GERDEMANN, J. W. and M. B. LINFORD 1953. A cyst-forming nematode attacking clover in Illinois. Phytopath., 43 (11), pp. 603～608.

GOFFART, H. 1941. Der Göttinger Erbsennematode (*Heterodera göttingiana*), ein Rückblick auf eine 50 jährige Entwicklung. Zbl. Bakt., Abt. 2, 104 (4/7), pp. 81～86.

GOFFART, H. 1951. Nematoden der Kulturpflanzen Europas. Paul Parey, Berlin. 144 pp.

GOODEY, T. 1937. Two methods of staining nematodes in plant tissues. Jour. Helminth., 15 (3), pp. 137～144.

GOODEY, T. 1951. Laboratory methods for work with plant and soil nematodes. Min. Agr. Fish., Technical Bulletin No. 2. 25 pp.

HIRSCHMANN, H. 1956. Comparative morphological studies on the soybean cyst nematode, *Heterodera glycines* and the clover cyst nematode, *H. trifolii* (Nematoda: Heteroderidae). Proc. Helminth. Soc. Wash., 23 (2), pp. 140～151.

HORI, S. 1915. Phytopathological notes. 5. Sick soil of soybean caused by a nematode. (in Japanese) Byôtyûgai-Zasshi, 2 (11), pp. 927～930.

ICHINOHE, M. 1952. On the soybean nematode, *Heterodera glycines* n. sp., from Japan. Ôyô-Dôbutsugaku-Zasshi, 17 (1/2), pp. 1～4.

ICHINOHE, M. 1953. On the parasitism of the soybean cyst nematode, *Heterodera glycines*. (in Japanese) Hokkaido Nat'l Agr. Expt. Sta. Res. Bul., 64, pp. 113～124.

ICHINOHE, M. 1955. Studies on the morphology and ecology of the soybean cyst nematode, *Heterodera glycines*, in Japan. (in Japanese) Hokkaido Nat'l Agr. Expt. Sta. Report, 48. 64 pp.

ICHINOHE, M. 1955 a. An experiment on the parasitism of the soybean cyst nematode by grafting. (in Japanese) Annual Rept. Soc. Pl. Prot. North Japan, 6, pp. 53～56.

ICHINOHE, M. 1955 b. A study on the population of the soybean cyst nematode (*Heterodera glycines*). I. An observation on the relation between the crop damage and the female infestation. (in Japanese) Hokkaido Nat'l Agr. Expt. Sta. Res. Bul., 68, pp. 67～70.

ICHINOHE, M. 1955 c. Survey on the "Yellow dwarf" disease of soybean plants caused by *Heterodera glycines* occurring in the peat soil in Hokkaido. (in Japanese) Jap. Jour. Ecology, 5 (1), pp. 23～26.

ICHINOHE, M. and K. ASAI 1956. Studies on the resistance of soybean plants to the nematode, *Heterodera glycines*. I. Varieties, "Daiichi-hienuki" and "Nangun-takedate". (in Japanese) Hokkaido Nat'l Agr. Expt. Sta. Res. Bul., 71, pp. 67～79.

ICHINOHE, M. 1959. Studies on the soybean cyst nematode *Heterodera glycines* and its injury to soybean plants in Japan. Pl. Dis. Repr., Supplement 260, pp. 239～248.

ISHIKAWA, T. 1916. Principal insect pests and diseases of plants occurring during the fourth year of Taisho. Occurrence of "Moon night" and wilt disease of soybean. (in Japanese) Byôtyûgai-Zasshi, 12 (9), pp. 497～508.

ITO, S. 1921. Studies on "Yellow dwarf" disease of soybeans. (in Japanese) Hokkaido Agr. Expt. Sta. Report, 11, pp. 47～59.

IWATA, K. 1941. A basic test on the control of "Yellow dwarf" disease of soybeans by rotations. (in Japanese) Nôgyô-oyobi-Engei, 16 (3), pp. 429～435.

JONES, F. G. W. 1954. First steps in breeding for resistance to potato-root eelworm. Ann. Appl. Biol., 41 (2), pp. 348~353.

KATSUFUJI, K. 1919. "Yellow dwarf" a new nematode disease of soybean. Ann. Phytopath. Soc. Japan, 1 (2), pp. 1~5.

KÔSAKA, T. 1950. On the resistance of sweet potato to the root lesion nematode. I. An anatomical study. (in Japanese) Kyûshû Agr. Res., 7, pp. 65~66.

LIEBSCHER, G. 1890. Eine Nematode als Ursache der Erbsenmüdigkeit des Bodens. Dtsch. landw. Pr., 17 (56), pp. 436~437; (61), p. 477; (84), p. 672.

LIEBSCHER, G. 1892. Beobachtungen über das Auftreten eines Nematoden an Erbsen. J. Landw., 40, pp. 357~368.

McBETH, C. W. 1954. Some practical aspects of soil fumigation. Pl. Dis. Repr., Supplement 227, pp. 95~97.

MIZOGAMI, T. 1947. An anatomical study on the resistance of sweet potato to the root-knot nematode. (in Japanese) Kyûshû Agr. Expt. Sta. Res. Meeting Abstract No. 1, pp. 19~20.

MANKAU, G. R. and M. B. LINFORD 1956. Soybean varieties tested as hosts of the clover cyst nematode. Pl. Dis. Repr., 40 (1), pp. 39~42.

MUKASA, K. and M. ICHIINOHE 1952. A study on the nematode-disease index to soybean varieties using relative index system. (in Japanese) Hokkaido Nat'l Agr. Expt. Sta. Res. Bul., 63, pp. 117~120.

Nagano Pref. Agr. Expt. Sta. 1952, 1954. Annual report on the insect pest control experiments. (not for publication)

NAKATA, K. and H. ASUYAMA 1938. Survey of the principal diseases of crops in Manchuria. (in Japanese) Manchuria Indust. Rept., 32. 166 pp.

OOSTENBRINK, M. 1952. Remarks in discussion in Proc. Int. Nemat. Symposium, Harpenden, 1951. F. A. O., Rome.

OOSTENBRINK, M. and H. DEN OUDEN 1954. De structuur van de kegeltop als taxnomisch kenmerk bij *Heterodera*-soorten met citroenvormige cysten. Tijdschr. Pl'Ziekt., 60 (3), pp. 146~151.

RASKI, D. J. 1950. The life history and morphology of the sugar-beet nematode, *Heterodera schachtii* SCHMIDT. Phytopath., 40 (2), pp. 135~152.

RASKI, D. J. 1954. Soil fumigation for the control of nematodes on grape replants. Pl. Dis. Repr., 38 (12), pp. 811~817.

ROSS, J. P. and C. A. BRIM 1957. Resistance of soybeans to the soybean cyst nematode as determined by a double-row method. Pl. Dis. Repr., 41 (11), pp. 923~924.

ROSS, J. P. 1958. Host-parasite relationship of the soybean cyst nematode in resistant soybean roots. Phytopath., 48 (10), pp. 578~579.

SHIBUYA, M. 1952. Studies on the varietal resistance of sweet potato to the root-knot nematode injury. Mem. Facul. Agr. Kagoshima Univ., 1 (1), pp. 1~22.

SKOTLAND, C. B., J. N. SASSER and N. N. WINSTEAD 1955. Preliminary reports of results of research on the soybean cyst nematode in North Carolina. Ann. Rept. Soybean Cyst Nema. Control., Pl. Pest Control. Branch, U. S. D. A., pp. 19~25.

SKOTLAND, C. B., N. N. WINSTEAD and J. N. SASSER 1956. The soybean cyst nematode disease. N. C. Agr. Ext. Folder 126.

SKOTLAND, C. B. 1957. Biological studies of the soybean cyst nematode. Phytopath., 47 (10),

pp. 623~625.

SMITH, A. L. and A. L. TAYLOR 1947. Field methods of testing for root-knot infestation. Phytopath., 37 (2), pp. 85~93.

TANAKA, T. 1921. On the soybean nematode. (in Japanese) Byôtyûgai-Zasshi, 8 (11), pp. 551~553.

THORNE, G. 1949. On the classification of the Tylenchida, new order (Nematoda, Phasmidia). Proc. Helminth. Soc. Wash., 16 (2), pp. 37~73.

WINSTEAD, N. N., C. B. SKOTLAND and J. N. SASSER 1955. Soybean cyst nematode in North Carolina. Pl. Dis. Repr., 39 (1), pp. 9~11.

Yamagata Pref. Agr. Expt. Sta. 1955. Annual Report. (not for publication)

YOKOO, T. 1936. Host plants of *Heterodera schachtii* SCHMIDT and some instructions. (in Japanese) Korea Agr. Expt. Sta. Bul., 8 (2/3), pp. 167~174.

YOKOO, T. 1951. Golden nematode and its relatives. (in Japanese) Ann. Phytopath. Soc. Jap., 15 (3/4), pp. 166~167.

摘　　要

ダイズシストセンチュウ (*Heterodera glycines*) に関する研究

農林技官　一　戸　　稔*

ダイズシストセンチュウ (*Heterodera glycines* ICHINOHE) は，わが国のおもなダイズ栽培地帯で大正初期以来大きな被害を与えている線虫で，堀正太郎 (1915) により福島県白河地方で初めて発見された。その後，新潟県 (石川滝太郎，1916) その他の県でも記録せられ，また北海道では勝藤孝一 (1919)，伊藤誠哉 (1921) によって伊達地方で初めてその発生が確認されている。わが国の有数なダイズ特産地である北海道にとって，ダイズシストセンチュウは各種病害虫のなかの最重要課題の一つで，この線虫の防除に関する研究が北海道農業試験場において大正年代以来続けられている。ここには筆者が主として 1949 年以降 1958 年までにおこなったダイズシストセンチュウの形態，分類，生態，防除に関する研究の概要を取りまとめて報告した。

1. ダイズシストセンチュウの幼虫は，細長く，体長が平均 471 ミクロンで，これが寄主植物の根の組織に侵入寄生し，組織内で脱皮を 3 回繰返して成虫となる。線虫卵の発生経過の途中に，卵殻内での幼虫の脱皮が 1 回認められるので，卵から孵化直後の幼虫は〝第 2 (齢) 幼虫〟と呼ばれる。幼虫の性を外観的に識別できるのは〝第 3 (齢)〟以降の幼虫で，このとき雌雄によって体内の生殖原基の発達の様相が異なる。雌成虫は表皮が肥厚褐変してシストとなる。シスト内の卵数は 95～478，平均 262 である。雌成虫の尾端に作られる卵嚢には 200 個以内の卵が排出される。

2. ダイズシストセンチュウは，堀正太郎 (1915) によって SCHACHT (1859) がドイツから報告した〝甜菜線虫〟(*Heterodera schachtii*) と同一種として初めて報告された。以来一般にはこの学名が用いられてきたが，筆者はヨーロッパ産の〝甜菜線虫〟の標本を取寄せてダイズシストセンチュウの形態と精細に比較した結果から，わが国の線虫を未記載の新種と認め，1952 年に *Heterodera glycines* と命名し記載した。本種は，シストの形態的特徴からは "*schachtii* group" と呼ばれる種群に属し，クローバーに寄生するシストセンチュウ (*Heterodera trifolii*) ―わが国にも分布する― に最もよく似ている。両種の区別点は，第 2 幼虫における口針基部から背部食道腺開口部までの長さによるのが普通である。

3. ダイズシストセンチュウの寄主植物であるダイズ，アズキ，インゲン，ハナマメの 4 種を試験枠に生育させ，根に寄生する雌成虫数を比較した結果，ダイズとアズキでは多数の雌虫を認めたが，インゲンではきわめて少なく，またハナマメでは全く雌虫を認めなかった。一方，ダイズ，アズキ，インゲンの各植物の間で，根に侵入する線虫幼虫の数，侵入後

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の幼虫の発育の速さなどを比較したところ，前記の3植物間に有意な差異がみとめられなかった。それにもかかわらず前記寄主植物間で根の寄生雌成虫数が異なった原因として，つぎのことが確かめられた。すなわちインゲンでは若い雌成虫のその後の発育がほとんど停滞し虫体の大きさや産卵数で比較するとダイズやアズキに発育した雌成虫よりも著しく劣り，またインゲンの根の表皮を破って根の外側に出るほど発達する成虫の数が明らかに少ない。さらにハナマメでは，第4幼虫まで発達した幼虫でその後に斃死するものが多く，雌成虫にまで発育できる個体はきわめてまれである。

4. これまでダイズシストセンチュウの非寄主植物とされている多数種の植物についてそれらに対する線虫の寄生性を調査した。ダイズシストセンチュウは，非寄主植物でもそのある種類とくにマメ科の種類に対し，根の組織に幼虫が侵入しうることが確かめられた。この場合侵入後の幼虫の発育は，何らかの原因で発育の起らない場合や，一旦生長し始めた幼虫が成虫になる前に斃死する場合など，植物の種類によって侵入後の幼虫の発育に差異が認められる。

5. 札幌地方におけるダイズシストセンチュウの年間の最大世代数を推定するために，野外に設置した枠により試験を8回繰返した。その結果，線虫の発育零点（発育限界低温度）が10°Cと算出された。さらに札幌地方における圃場のダイズ生育期間中の深さ別の日平均地温，同期間中の発育有効積算温度，線虫の1世代に必要な発育有効積算温度などを算出し，これらの数値から年間世代数が3回以内であることを推定した。

6. ダイズほか数種の寄主植物および非寄主植物を用い，種間および属間の接木を〝呼接法〟によって試みた。接木が成功したのは，インゲンを台木にライマビーンを接穂にした場合だけであった。このインゲンの根に接がれたライマビーンの9個体を線虫土壌に移し，ダイズシストセンチュウが寄生した場合の根の組織内の線虫の発育状況とくに雌成虫の大きさ，産卵数などを調査した。線虫の発育は接木によらない正常のインゲンの根に寄生した場合とほとんど差異なく，したがって接穂が非寄主植物（ライマビーン）であっても，根に寄生した線虫に対してその影響をおよぼしがたいことが確かめられた。

7. 収穫時のダイズにおける線虫被害と土壌中の線虫密度との関連を調査した結果，両者の間に明らかな負の相関々係が成立する。

8. 寄主植物，非寄主植物を栽培することによって起こる作付前後の土壌中の線虫密度の変動について調査した。ダイズおよびアズキ以外の供試植物は，それぞれの1回の作付により土壌中の線虫密度をかなり低下させる。とくにインゲンは，寄主植物の一つであるにもかかわらず，1作栽培することにより，他の非寄主植物の栽培または休閑の場合よりも大きく土壌中の線虫密度を低下させる。

9. 1927年以来ダイズを連作している連作圃場試験区に，1955年に初めてダイズシストセンチュウによる病徴が観察されたので，同年より3年間，区内のダイズ収量と土壌中の線虫密度の変動を継続して調査し，両者が高い負の相関々係を示すことを確かめた。とくにダイズの草丈・総重または子実重と収穫時の土壌中の線虫密度（土壌10g中のシスト数で表わされる）とは最も高い相関を示し，子実重と線虫密度との関係を示す回帰式を求めると，

Y＝14.1－0.233 X であった。

10. ダイズ品種のダイズシストセンチュウに対する抵抗性に関して，いわゆる抵抗性品種〝第1稗貫〟および〝南部竹館〟，罹病性品種〝十勝長葉〟および〝克霜〟を用いて試験を行なった。供試した両抵抗性品種は熟期が極端におそいため北海道では一般圃場に用いえない。これらの品種に，ガラス室内で短日処理を施して早期に成熟させ，生育および収量を調査したが，両品種の線虫に対する〝耐虫性〟は短日処理によっても失なわれない。一方，根に侵入する線虫幼虫の数では供試4品種間に差異が認められないが，侵入後の幼虫の発育が品種間で異なり，耐虫性の品種ほど侵入後の第2幼虫で斃死する数が多い。根の形態，生態を供試品種間で比較すると，耐虫性の品種ほど根系が大きく，側根が太く，根の単位重量当りの雌成虫寄生数が少なく，またバクテリヤによる根瘤数が根の単位重量当りで多い。また同一品種では根の雌成虫寄生数とバクテリヤ根瘤着生数との間に負の関係が認められ，このことから線虫と根瘤バクテリヤとの間の拮抗現象が推察される。要するに，次ぎの諸要因はダイズ品種のダイズシストセンチュウ抵抗性と深い関連があると結論される。

(1) 根に侵入した幼虫を早期に斃死させる要因

(2) 根瘤バクテリヤの活動をさかんにする要因

(3) 根の生育，ひいては植物体全体の生育をさかんにする要因

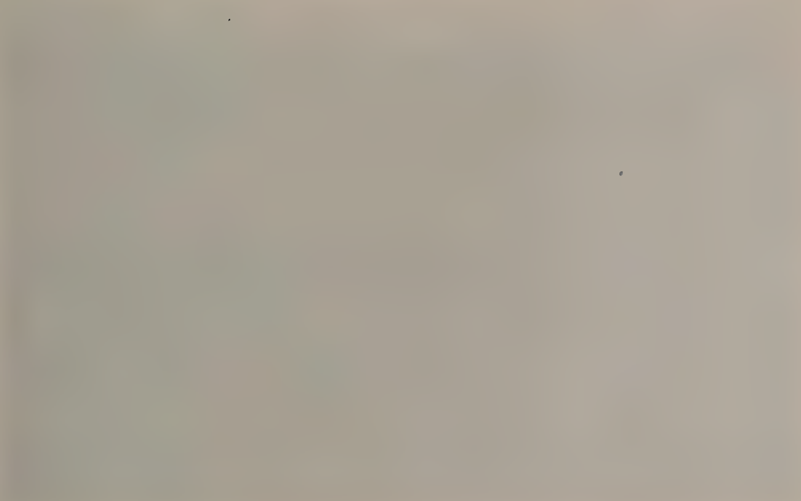
なお供試品種〝第1稗貫〟および〝南部竹館〟は，真に〝抵抗性〟ではなく〝耐虫性〟であると解される。

11. ダイズシストセンチュウの防除法として，抵抗性ないし免疫性植物を組入れた輪作が第1に推奨される。マメ科のレッドクローバー，アルファルファ，エンドウなどをこの目的で用いると，他の種類の作物の栽培または休閑の場合に比べて土壌中の線虫密度を低下させる度合が大きく，明らかにその効果が期待できる。なおインゲンは本線虫の寄主植物の一つとされているが，その作付によりかえって土壌中の線虫密度を低下させ，輪作に組入れる〝捕獲作物〟として用いうる。

12. 抵抗性のダイズ品種を栽培することはダイズシトセンチュウの防除に有効であるが既存の耐虫性品種はいずれも熟期がきわめておそく，また子実の品質の不良なものが少なくないので，今後これらの点を改良した〝抵抗性〟新品種の育成が望まれる。この場合，1956年に供試したダイズ品種〝Laredo〟は，根の雌成虫寄生数では供試した他のいずれの耐虫性品種よりも極端に少なく，この品種のこの特性はさらに検討に値すると思われる。

13. ダイズシストセンチュウの化学的防除法として，各種の薬剤を用いたポット試験，圃場試験の成績から，ヂクロールプロペン(〝D-D〟)および二臭化エチレン(〝ネマヒューム〟)が本線虫の防除に最も有効である。一般に用いうる方法は，両薬剤とも10 a 当り 20～30 l を 30 cm 間隔，深さ 15 cm の土壌中に注入する方法である。

14. 化学薬剤によるダイズシストセンチュウ防除の現状は，薬価，薬剤を土壌に処理する機械の発達，栽培条件および気象条件にもとづく薬剤施用時期の制約，大豆作自体の低収益性などの諸点からみて，なお問題が多い。将来，植物に薬害をおよぼさない廉価な殺線虫剤の出現がダイズの栽培地帯では渇望されている。



Explanation of Plate I

A A soybean field affected by the soybean cyst nematode.
Photographed on September 17th, 1960 at Komoro, Nagano Prefecture.

B Soybean plants affected by the soybean cyst nematode.
Photographed on September 15th, 1960 at Kikyôgahara, Nagano Prefecture.

A

B

M. ICHINOHE photo





Explanation of Plate II

A Molting of the first-stage larva in egg-shell.

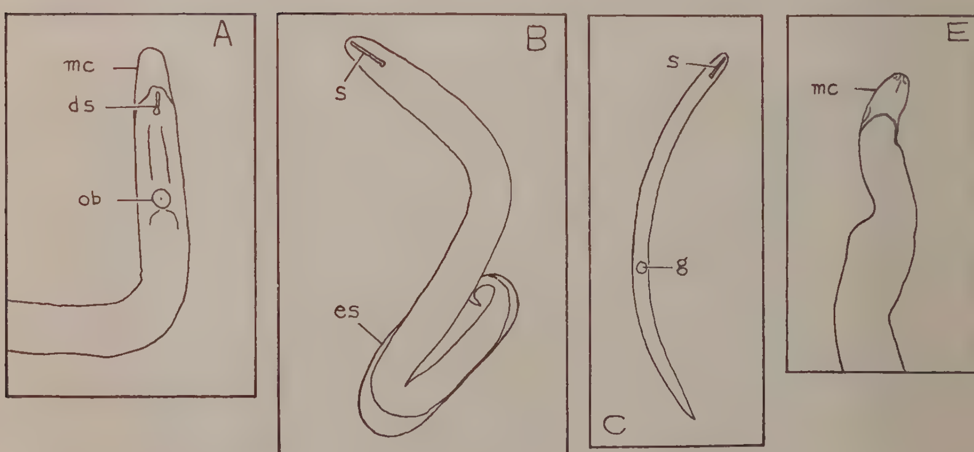
B The second-stage larva hatching from egg.

C The second-stage larva.

D The second-stage larva further developed in root tissue.

E Anterior portion of the molting second-stage larva in root tissue.

ds : developing stylet
es : egg-shell
g : genital primordium
mc : molted cuticle
ob : developing oesophageal bulb
s : stylet



A

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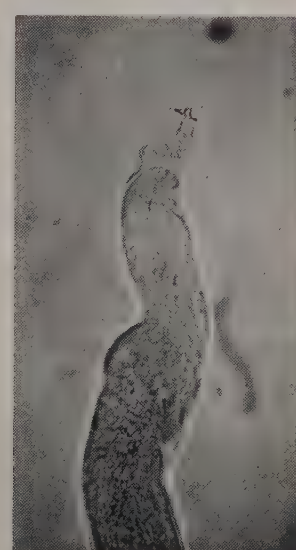


E

M. ICHINOHE photo

Explanation of Plate III

A The third-stage larva.

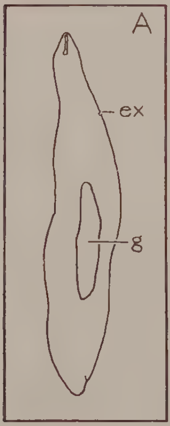
B The molting third-stage male larva (early stage of molting).

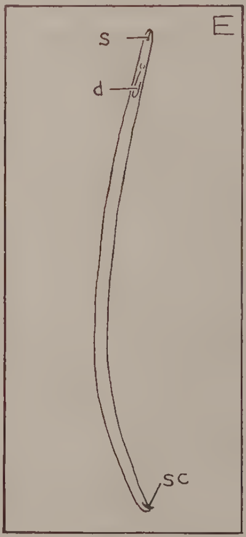
C The molting third-stage male larva (mid-stage of molting).

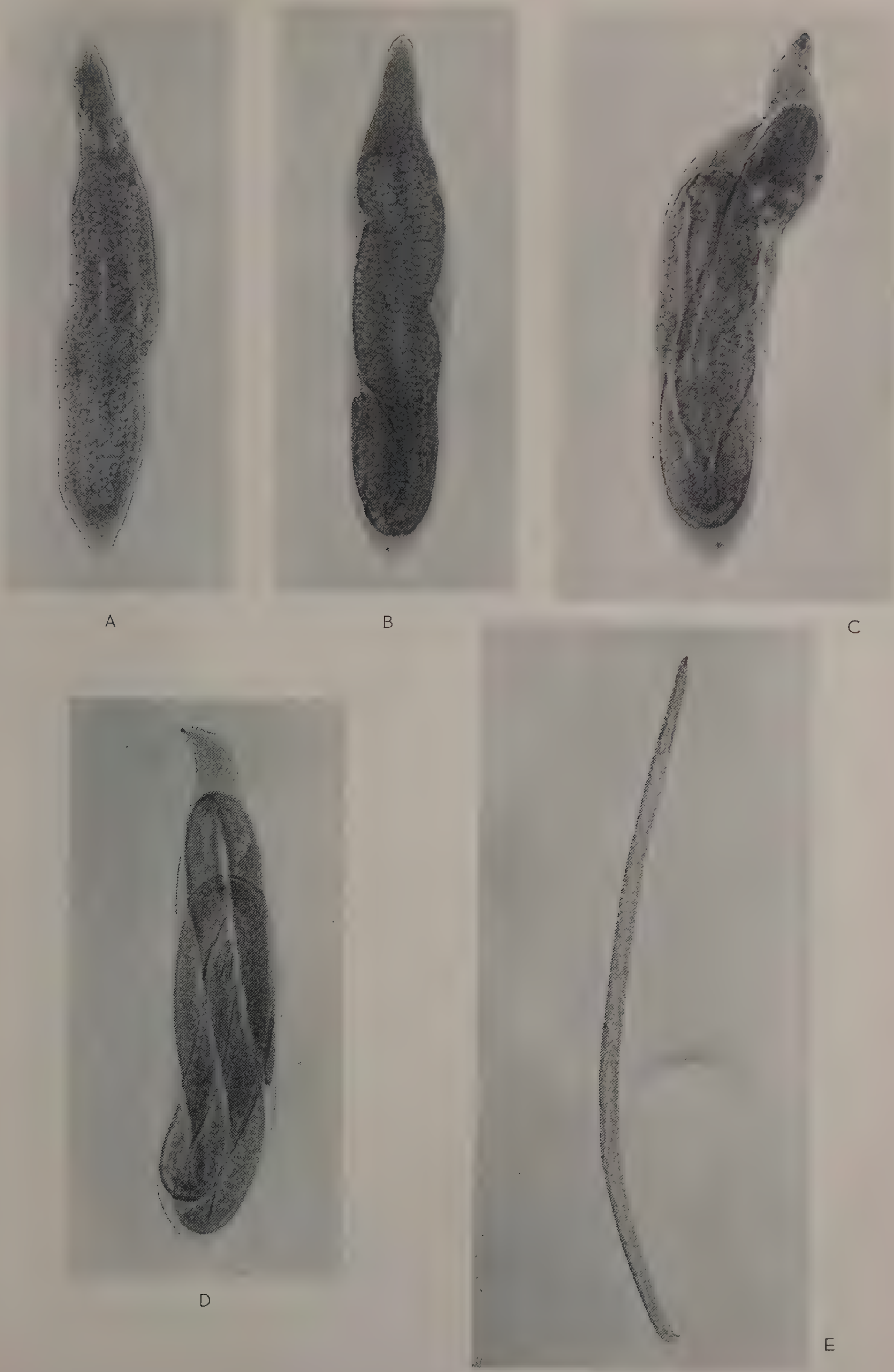
D The adult male coiled in the third-stage larval skin.

E The adult male.

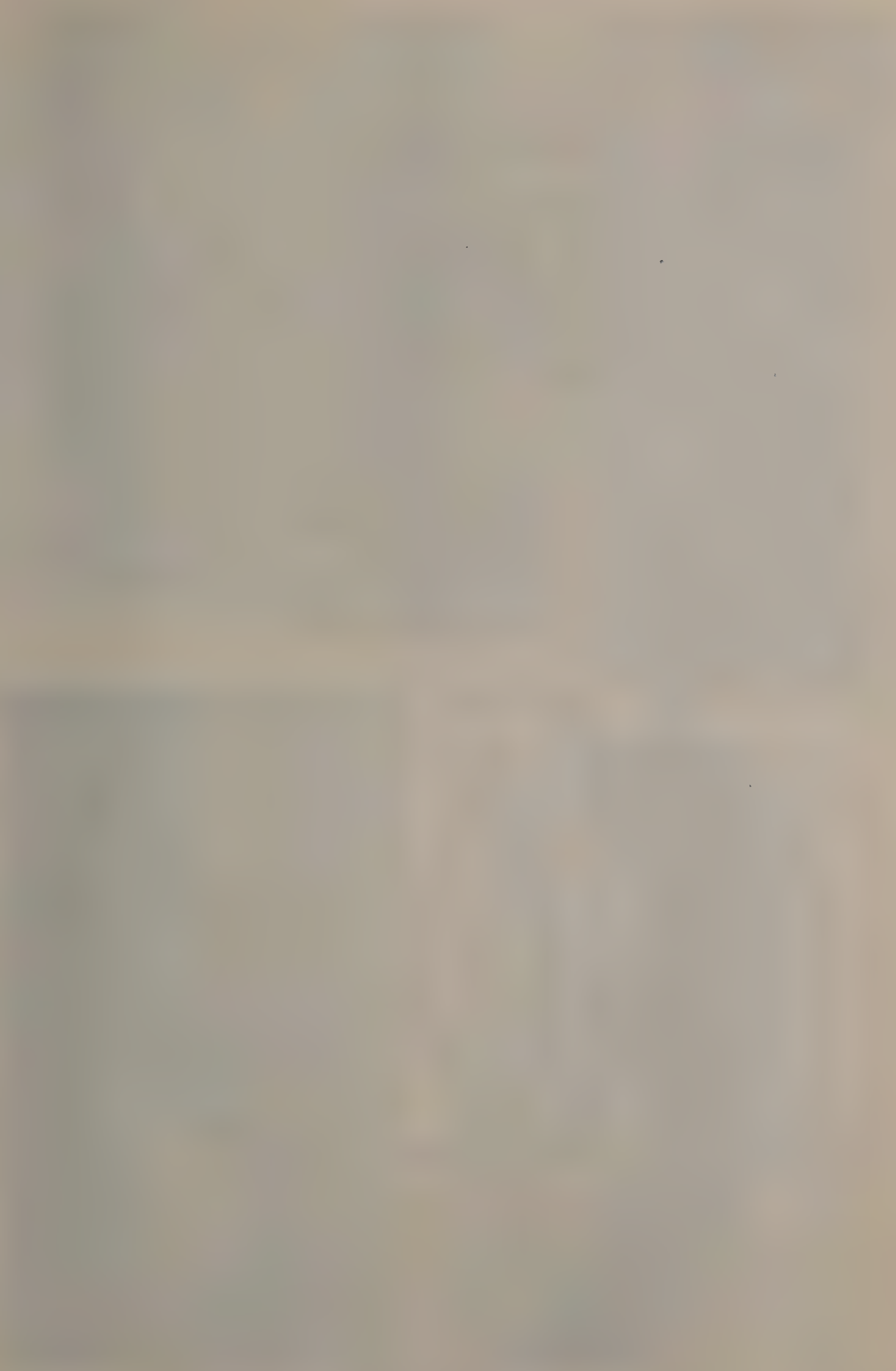
d : dorsal oesophageal gland
ex : excretory pore
g : genital primordium
s : stylet
sc : spicules







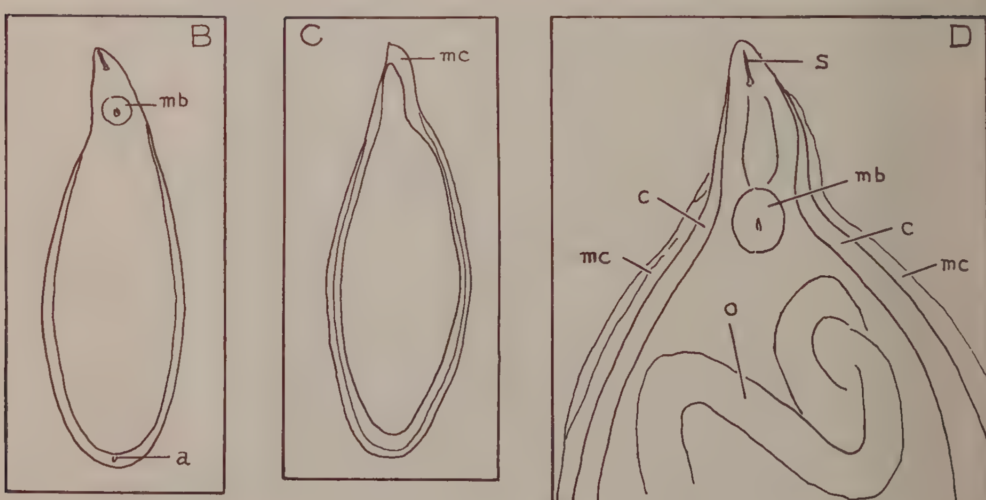
M. ICHINOHE photo



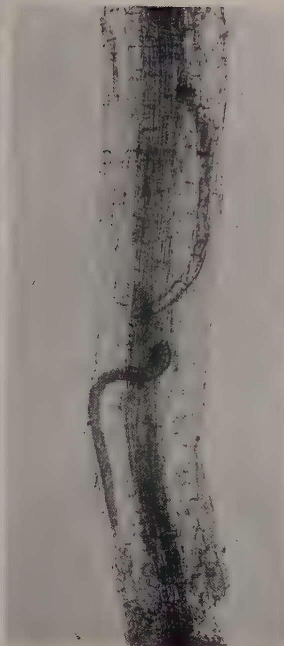
Explanation of Plate IV

A	The molting third-stage female larva.
B	The fourth-stage female larva.
C	The molting fourth-stage female larva.
D	Anterior portion of the adult female.
E	The adult female.

a :	anus
c :	cuticle
mb :	medium oesophageal bulb
mc :	molted cuticle
o :	ovary
s :	stylet



A



B

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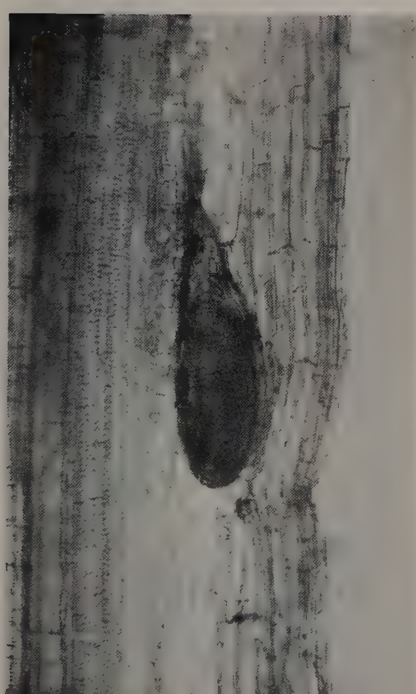
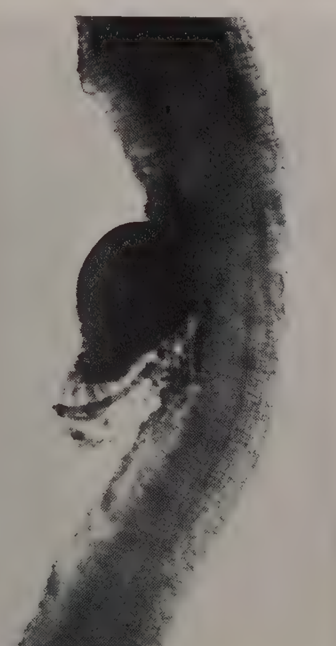


D



E

M. ICHINOHE photo

Explanation of Plate V

A Transverse section of soybean root.

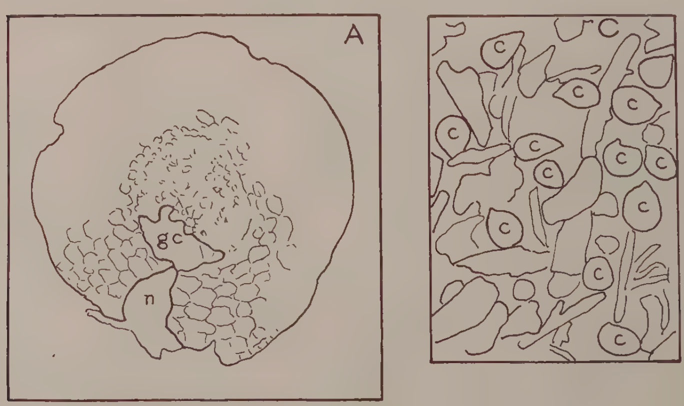
B Longitudinal section of soybean root having the nematode cyst which is filled with eggs.

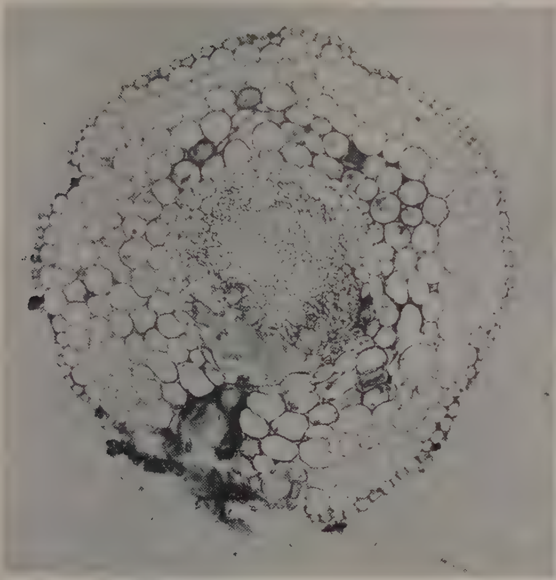
C The cysts extracted from soil.

D Zig-zag pattern on the surface of cyst wall.

E The "punctations" on the cyst wall.

c : cyst
gc : giant cell
n : anterior portion of the female nematode





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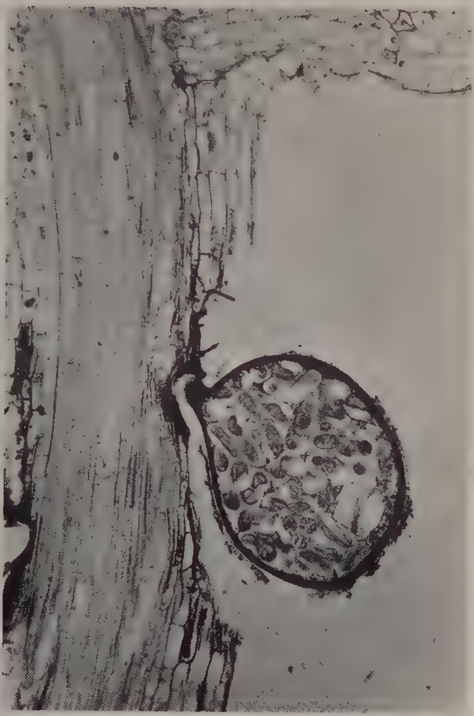
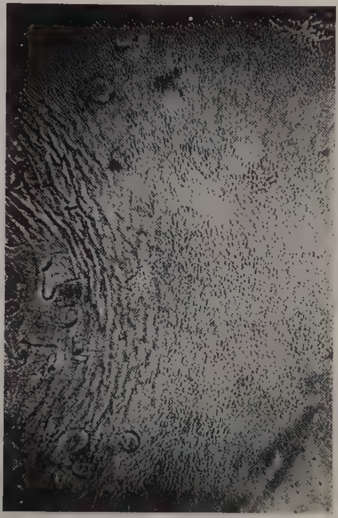


B

C

D



E

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Explanation of Plate VI

A The second-stage larvae invading soybean root.

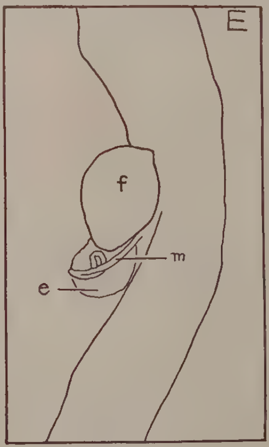
B The second-stage larvae located in soybean root tissue.

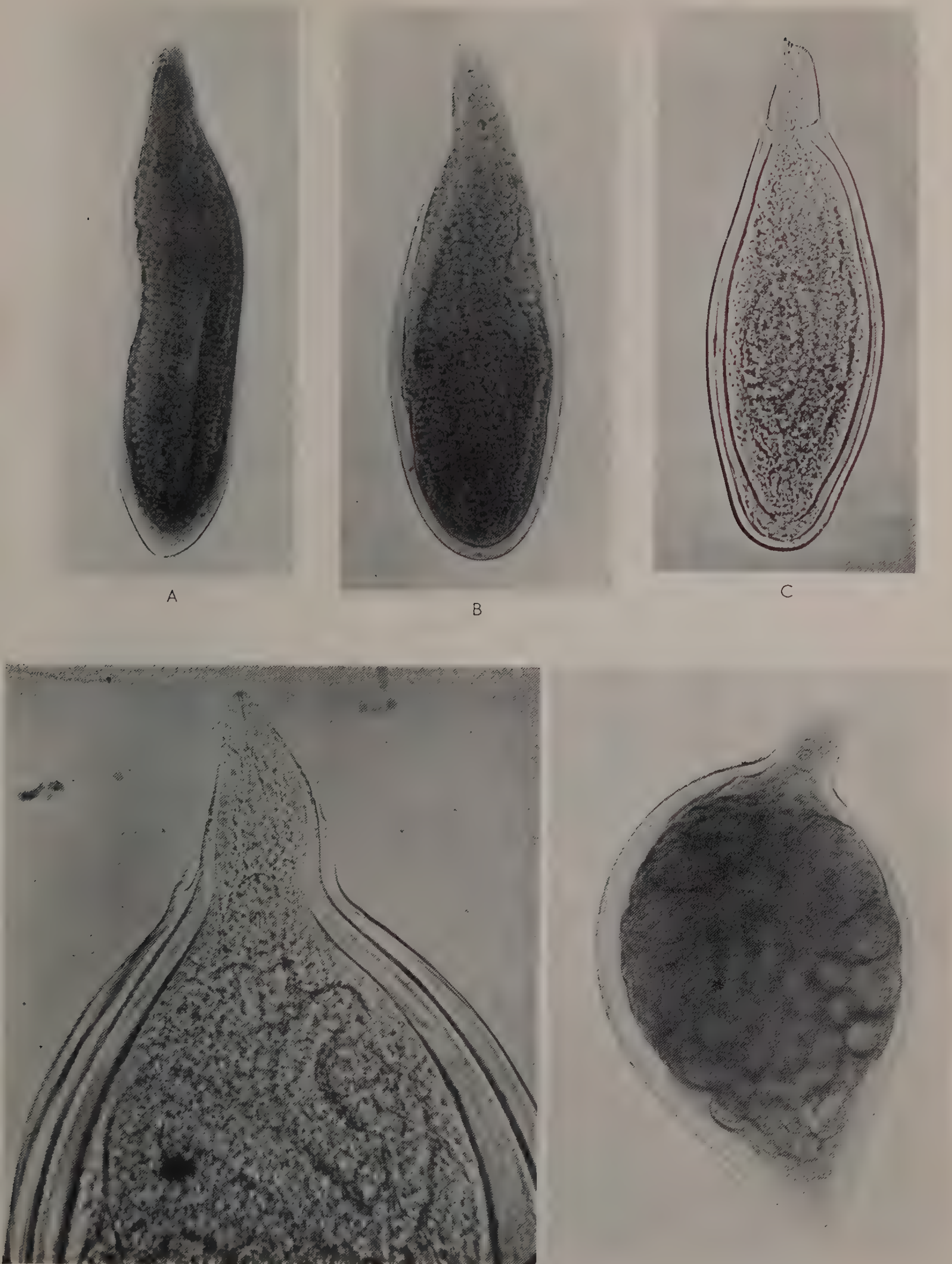
C The developing second-stage larva in soybean root tissue.

D The fourth-stage female larva in soybean root tissue.

E The adult female attached to soybean root.

e : egg sac
f : female nematode
m : male nematode
n : larval nematode





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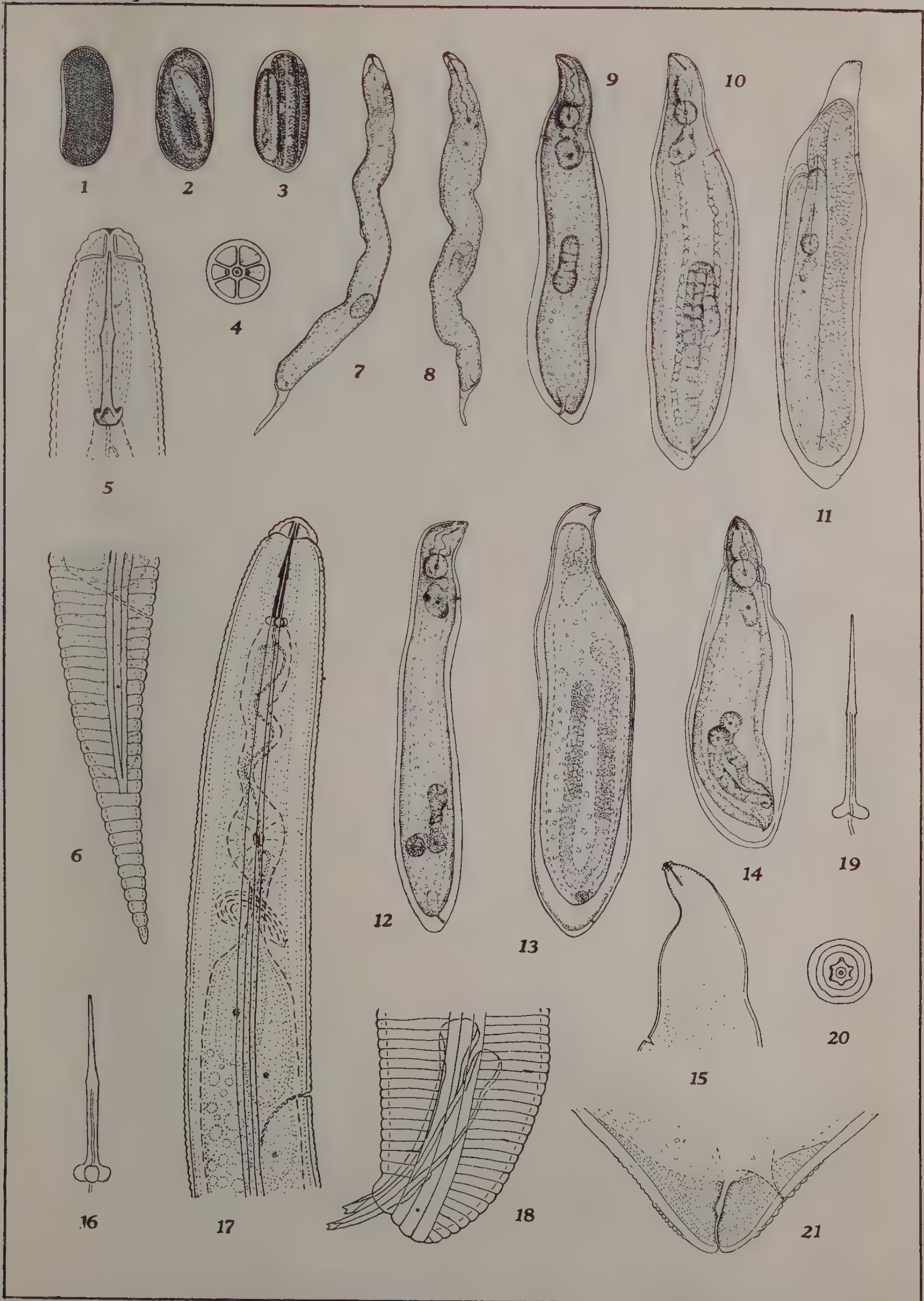
Explanation of Plate VII

1∼3. Development of the second-stage larva in egg-shell.
4. Face view of the second-stage larva.
5. Anterior portion of the second-stage larva.
6. Posterior region of the second-stage larva.
7, 8. The molting second-stage larvae.
9. The third-stage male larva.
10. The third-stage male larva showing cellular string in lateral regions.
11. The molting fourth-stage male larva.
12. The third-stage female larva.
13. The molting third-stage female larva.
14. The molting fourth-stage larva.
15. Anterior portion of the third-stage larval skin.
16. Stylet of the adult male.
17. Anterior portion of the adult male.
18. Posterior portion of the adult male.
19. Stylet of the adult female.
20. Face view of the adult female.
21. Posterior region of the adult female.



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